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NOT FOR PUBLICATION

COPY NO. 1

NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
OF THE  
SEVENTH MEETING  
OF THE  
EXECUTIVE  
OF THE  
ASSOCIATE COMMITTEE  
ON DENTAL RESEARCH

OTTAWA

17 OCTOBER, 1949







Proceedings of the Seventh Meeting

of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

4. Fin Held in Room 311, Chateau Laurier, 8 p.m., 17 October, 1949.

1. Attendance

Dr. R. G. Ellis, Chairman

Dr. E. Charron

Mr. S. J. Cook, Secretary

(Dr. D.L. Thomson and Col. E.M. Wansbrough were unable to attend because of other engagements, but were present at the meeting of the Committee 18 October when these proceedings were reviewed.)

By invitation

Dr. J. S. Bagnall

2. Dr. Bagnall's Report Under DR 7

Consideration was given to the method of publishing the summary on caries research which had been presented by Dr. Bagnall at the 10th Meeting of the Associate Committee.

It was AGREED that estimates should be secured by the Secretary on the cost of reproducing this summary including the bibliography in a document about 6 by 9 inches and in an edition of 5,000 copies with cover. Costs were to be obtained on reproduction by lithomat and also by printing. It was AGREED also to include a foreword indicating the need for the survey and reference to the main report, and the disposition of copies of it. It was thought also that a short account of the work of the Associate Committee should be included. The Secretary was asked to draft this document and to submit it to the Executive and to Dr. Bagnall for consideration.

3. Main Report Including Bibliography on Dental Caries

The Executive was of the opinion that the complete report including the bibliography would be invaluable as a reference in dental libraries and after considerable discussion on the subject it was agreed to get data on the cost of stencilling 25 to 30 copies on 8 1/2 by 11 inch paper in



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the form as presented by Dr. Bagnall except that the text should preferably be single spaced and printed on both sides of the page. It was thought the book should be bound in serviceable covers. It was also suggested that the cost of micro-filming the entire document for possible wider distribution should also be secured.

#### 4. Finances

THE CHAIRMAN reported that certain applications had been received for additional grants. These were dealt with as follows:-

(i) DR 18 - Dr. H. K. Box

Owing to an accumulation of arrears in the matter of payments and vouchers, Dr. Box's grant under DR 18 requires \$458.80 additional this year in order to balance his account as of 31 March, 1950.

It was AGREED to recommend this payment.

(ii) DR 30 - Dr. Norma Ford Walker

A grant had been made for a period of six months at \$200 a month under DR 30 with the understanding that the applicant might make a further application if the need arose. Dr. Walker has sufficient funds to carry her work to December 1949 but requires an additional grant of \$600 for the months January - March 1950.

It was AGREED to recommend this grant.

(iii) DR 11 - Dr. R. J. Godfrey

This grant in the amount of \$2200 had been made for the year 1948-49 but owing to a delay in returning vouchers for some of the expenditures made in that year there is now a deficit of \$559.07. Vouchers for this amount were received in the present fiscal year but were chargeable to the preceding fiscal year.

It was AGREED to recommend payment of this amount.

#### 5. Applications for Grants

THE CHAIRMAN announced that new application forms had been prepared and that the deadline for their return was 1 February, in each year. It was AGREED that application forms should be





sent out to grantees and to the universities at an early date. In connection with fellowship forms it was agreed to send only half the number mailed last year but to retain the same distribution list. The Secretary was instructed to send a covering letter with the new fellowship forms asking that all previous forms be destroyed.

## 6. Publicity

(i) THE EXECUTIVE recommends that a summary of the proceedings of each meeting be prepared for publication in the Journal of the Canadian Dental Association.

(ii) THE EXECUTIVE also recommends that copies of the proceedings of all meetings of the Committee be sent to deans of dental schools who are not members of the Associate Committee.

## 7. Date of Next Meeting

It was AGREED that the next meeting of the Committee should be held in February at the call of the Chair.

The Meeting adjourned at 10 P.M.

- |                                |                |
|--------------------------------|----------------|
| 10. Dr. G. H. Best             | 31 March, 1950 |
| 11. Dr. J. B. Collier          | 31 March, 1950 |
| 12. Dr. G. W. Ellis            | 31 March, 1950 |
| 13. Dr. J. E. W. Ferguson      | 31 March, 1950 |
| 14. Dr. A. W. Ham              | 31 March, 1950 |
| 15. Dr. J. J. R. R. R.         | 31 March, 1950 |
| 16. Dr. G. E. Stewart          | 31 March, 1950 |
| *17. Dr. D. B. Thomson         | 31 March, 1950 |
| *18. Mr. S. J. Cook, Secretary |                |

### (18) To Other Persons:

20-22 Deans of Dental Schools (who are not members of the Committee)

- 20. Alberta - Dr. H. B. Hamilton
- 21. Saskatchewan - Dr. J. Stanley Bennett
- 22. McGill - Dr. D. Brewster's Wherry

- 23. Master Copy (General Secretary)
- 24. Board Room Copy
- 25. Library

26-30 Reserve.

\* Members of the Executive





## INITIAL DISTRIBUTION

### (i) To Members of the Committee

#### Associate Committee on Dental Research

|                                                                               | <u>Term to Expire</u> |
|-------------------------------------------------------------------------------|-----------------------|
| * 1. Dr. R. G. Ellis, <u>Chairman</u>                                         | 31 March, 1951        |
| 2. President C. J. Mackenzie (ex officio)                                     | --                    |
| <u>Rep. Dental Profession</u>                                                 |                       |
| 3. Dr. H. K. Box                                                              | 31 March, 1952        |
| * 4. Dr. E. Charron                                                           | 31 March, 1951        |
| 5. Dr. M. H. Garvin                                                           | 31 March, 1950        |
| 6. Dr. H. R. MacLean                                                          | 31 March, 1950        |
| 7. Dr. R. H. McDougall                                                        | 31 March, 1952        |
| <u>Rep. Royal Canadian Dental Corps</u>                                       |                       |
| * 8. Col. E. M. Wansbrough (ex officio)                                       | --                    |
| <u>Rep. Division of Dental Health</u><br><u>Dept. Nat. Health and Welfare</u> |                       |
| 9. Dr. H. K. Brown                                                            | --                    |
| <u>Rep. Basic and Med. Sciences</u>                                           |                       |
| 10. Dr. C. H. Best                                                            | 31 March, 1950        |
| 11. Dr. J. B. Collip                                                          | 31 March, 1952        |
| 12. Dr. O. W. Ellis                                                           | 31 March, 1951        |
| 13. Dr. J.K.W. Ferguson                                                       | 31 March, 1951        |
| 14. Dr. A. W. Ham                                                             | 31 March, 1952        |
| 15. Dr. J. J. Rae                                                             | 31 March, 1950        |
| 16. Dr. C. B. Stewart                                                         | 31 March, 1951        |
| * 17. Dr. D. L. Thomson                                                       | 31 March, 1950        |
| * 18. Mr. S. J. Cook, <u>Secretary</u>                                        | --                    |

### (ii) To Other Persons:

20-22 Deans of Dental Schools (who are not members of the Committee)

- 20. Alberta - Dr. W. S. Hamilton
- 21. Dalhousie - Dr. J. Stanley Bagnall
- 22. McGill - Dr. D. Prescott Mowry

- 23. Master Copy (General Secretary)
- 24. Board Room Copy
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NATIONAL RESEARCH COUNCIL OF CANADA

UNIVERSITY OF TORONTO  
FACULTY OF DENTISTRY  
OFFICE OF THE DEAN

RECD. DEC 8-1949

ANSWERED \_\_\_\_\_  
REFERRED TO \_\_\_\_\_  
FILE \_\_\_\_\_

PROCEEDINGS  
OF THE  
TENTH MEETING  
OF THE  
ASSOCIATE COMMITTEE  
ON DENTAL RESEARCH

OTTAWA

17-18 OCTOBER, 1949







## TABLE OF CONTENTS

|                                                   | <u>Page</u> |
|---------------------------------------------------|-------------|
| 1. Attendance .....                               | 1           |
| 2. Chairman's Remarks .....                       | 1           |
| 3. Minutes of the 9th Meeting .....               | 2           |
| 4. DR 1 - Dr. J. J. Rae .....                     | 2           |
| 5. DR 2 - Dr. G.H.W. Lucas .....                  | 2           |
| 6. DR 3 - Dr. H. K. Box .....                     | 3           |
| 7. DR 4 - Dr. H. K. Box .....                     | 3           |
| 8. DR 5 - Dr. R. L. Twible .....                  | 3           |
| 9. DR 6 - Col. E. M. Wansbrough .....             | 3           |
| 10. DR 7 - Dr. J. S. Bagnall .....                | 3           |
| 11. DR 8 - Dr. A.D.A. Mason .....                 | 4           |
| 12. DR 9 - Drs. O.J.Walker and H.R. MacLean ..... | 5           |
| 13. DR 10 - Dr. G. M. MacDonald .....             | 5           |
| 14. DR 11 - Dr. R. J. Godfrey .....               | 6           |
| 15. DR 12 - Dr. A. E. R. Westman .....            | 6           |
| 16. DR 13 - Dr. M. Doreen Smith .....             | 6           |
| 17. DR 14 - Dr. M. Doreen Smith .....             | 7           |
| 18. DR 15 - Dr. Norma Ford Walker .....           | 7           |
| 19. DR 16 - Dr. C. H. Best .....                  | 7           |
| 20. DR 17 - Dr. R. F. Shaner .....                | 7           |
| 21. DR 18 - Dr. H. K. Box .....                   | 8           |
| 22. DR 19 - Dr. J.K.W. Ferguson .....             | 8           |
| 23. DR 20 - Dr. Jules Thébaud .....               | 8           |
| 24. DR 21 - Dr. A. E. R. Westman .....            | 9           |
| 25. DR 22 - Dr. C. H. Best .....                  | 9           |
| 26. Meeting of the Executive .....                | 10          |







# TABLE OF CONTENTS (cont'd)

- 2 -

|                                                                    | <u>Page</u> |
|--------------------------------------------------------------------|-------------|
| 27. Attendance (Second Session) .....                              | 10          |
| 28. DR 23 - Dr. C. P. Leblond .....                                | 10          |
| 29. DR 24 - Dr. C.H.M. Williams .....                              | 11          |
| 30. DR 25 - Dr. A. E. R. Westman .....                             | 11          |
| 31. DR 26 - Dr. R. E. Moyers .....                                 | 11          |
| 32. DR 27 - Dr. J.W. Abbiss and Dr. A.G. Nutlay...                 | 11          |
| 33. DR 28 - Dr. O. J. Walker .....                                 | 12          |
| 34. DR 29 - Dr. M. Doreen Smith .....                              | 12          |
| 35. DR 30 - Dr. Norma Ford Walker .....                            | 12          |
| 36. Method of Tooth Repair .....                                   | 13          |
| 37. Report of Subcommittee on Publications .....                   | 14          |
| 38. Membership of the Committee .....                              | 15          |
| 39. Finances .....                                                 | 15          |
| 40. Fellowships .....                                              | 15          |
| 41. Air Travel .....                                               | 16          |
| 42. Attendance of Grantees at Meetings of the Com-<br>mittee ..... | 17          |
| 43. Report of the Executive .....                                  | 17          |
| 44. Date of Next Meeting .....                                     | 17          |

## Appendices

- Appendix "A" Improved methods for repairing methacrylate dentures, by Dr. A.E.R. Westman, DR 21.
- Appendix "B" Investigation of the use of methylmethacrylate for direct intra-oral fillings, by Dr. A.E.R. Westman, DR 25.
- Appendix "C" An investigation of the mechanism of repair of the supporting structures of the teeth, by Dr. C. H. Best, DR 22.
- Appendix "D" A comparison of local anaesthetics for conduction block, by Dr. J.K.W. Ferguson, DR 19.







TABLE OF CONTENTS (cont'd)

- 3 -

Page

|              |                                                                                                                                                                                              |
|--------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Appendix "E" | Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon, by Dr. C.P. Leblond, DR 23.                                                   |
| Appendix "F" | Investigation of cements for methacrylate inlays, by Dr. A.E.R. Westman, DR 12.                                                                                                              |
| Appendix "G" | A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches, by Dr. N.F. Walker, DR 15.                                                 |
| Appendix "H" | A histological study of tooth germ development both intra- and extra- alveolar with a survey of pathological changes in the developing tooth, by Dr. J.W. Abbiss and Dr. A.G. Nutlay, DR 27. |
| Appendix "I" | Study of closure of space following premature loss of deciduous teeth, by Dr. Norma Ford Walker, DR 30.                                                                                      |
| Appendix "J" | Measurement of physiologic forces in the mouth, by Dr. R. E. Moyers, DR 26.                                                                                                                  |
| Appendix "K" | An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry, by Dr. O.J. Walker and Dr. H. R. MacLean, DR 9.                                |
| Appendix "L" | Determination of small amounts of fluorine by means of the photoelectric colorimeter, by Dr. O.J. Walker, DR 28.                                                                             |
| Appendix "M" | The effects of hard and soft diets on the supporting structures of the teeth, by Dr. C.H.M. Williams, DR 24.                                                                                 |
| Appendix "N" | The experimental artificial production of dental caries in vitro, by Dr. H. K. Box, DR 18.                                                                                                   |
| Appendix "O" | Chemical mechanisms in dental caries, by Dr. J.J. Rae, DR 1.                                                                                                                                 |
| Appendix "P" | Biological absorption of fluorine, by Dr. M. Doreen Smith, DR 29.                                                                                                                            |
| Appendix "Q" | A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario, by Dr. M. Doreen Smith, DR 13.                                    |





## TABLE OF CONTENTS (cont'd)

- 4 -

- Appendix "R" Improvement of sub-gingival curettage and gum resection techniques, by Dr. Jules Thébaud, DR 20.
- Appendix "S" A study of alveolar oxygen tension and blood oxygen content during nitrous oxide-oxygen anaesthesia, by Prof. G.H.W. Lucas, DR 2.
- Appendix "T" Trends in Caries Research, by Dr. J.S. Bagnall, DR 7.
- Appendix "U" National Research Council, Associate Committee on Dental Research, Grantees or Assistants invited to report personally (Table).
- Appendix "V" Policy of the Associate Committee on Dental Research regarding publications and reports (as adopted by the Committee) Min. 37:10th Meeting, 18/10/49.

### Initial Distribution

Dr. E. W. Anderson  
Dr. J. J. Cox  
Dr. D. L. Hargrave  
Col. E. M. Wainwright

Mr. E. J. Cook, Secretary

### By Invitation

Dr. J. S. Bagnall  
Mr. D. W. Christie  
Dr. W. J. Linghouse  
Dr. G.H.W. Lucas

Apologies for their absence were received from Dr. G. H. Hart, Dr. A. E. Ryan, Dr. C. F. Ellis, Dr. J.H.W. Ferguson and Dr. H. B. Maclean.

### 2. Chairman's Remarks

THE CHAIRMAN welcomed Dr. J.S. Bagnall, who had come to report on project DR 7; Mr. D. W. Christie from the Dental Research Foundation, present to report on DR 5, 14 and 21; Dr. W. J. Linghouse to present the report on DR 22; and Dr. G.H.W. Lucas to report on DR 2. He said that Dr. L.B. Hargrave of the University of Illinois would be present at the Second Session to report on Dr. Linghouse's grant under DR 21.





NATIONAL RESEARCH COUNCIL

Proceedings

of the

TENTH MEETING

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,  
9:30 a.m., 17-18 October, 1949.

1. Attendance

Dr. R. G. Ellis, Chairman  
Dr. H. K. Box  
Dr. E. Charron  
Dr. J. B. Collip  
Dr. M. H. Garvin  
Dr. A. W. Ham  
Dr. C. J. Mackenzie  
Dr. R. M. McDougall  
Dr. J. J. Rae  
Dr. D. L. Thomson  
Col. E. M. Wansbrough

Mr. S. J. Cook, Secretary

By Invitation

Dr. J. S. Bagnall  
Mr. D. R. Christie  
Dr. W. J. Linghorne  
Dr. G.H.W. Lucas

Apologies for their absence were received  
from Dr. C. H. Best, Dr. H. K. Brown, Dr. O. W. Ellis,  
Dr. J.K.W. Ferguson and Dr. H. R. MacLean.

2. Chairman's Remarks

THE CHAIRMAN welcomed Dr. J.S. Bagnall, who had come to report on project DR 7; Mr. D. R. Christie from the Ontario Research Foundation, present to report on DR 5, 12 and 21; Dr. W. J. Linghorne to present the report on DR 22; and Dr. G.H.W. Lucas to report on DR 2. He said that Dr. L.S. Bélanger of the University of Ottawa would be present at the Second Session to report on Dr. Leblond's grant under DR 23.





3. Minutes of the 9th Meeting

It was MOVED by Dr. Charron and SECONDED  
by Dr. Box:

That the Minutes of the 9th Meeting  
of the Committee, having been circulated, be  
taken as read and approved. CARRIED.

THE CHAIRMAN said that there were a few items of business arising  
out of the Minutes which would be dealt with under their res-  
pective items later in the Meeting.

Reports of Grantees

4. DR 1 - Dr. J. J. Rae

"An investigation of the chemical mechanisms  
involved in decrease of dental caries by fluorides"

DR. RAE presented a summary and an interim report on work done  
under his grant. These two documents appear in APPENDIX "O"

THE CHAIRMAN said that the question of publication of results  
would be dealt with later in the Meeting when the report of  
the Subcommittee on Publications was considered.

THE CHAIRMAN asked Dr. Rae whether his work on ammonia production  
in saliva tied in with the claims made for ammoniated dentifrices.

DR. RAE replied that he thought there was something in it.

DR. BOX observed that the periodontal mouth has no caries  
and is normally slightly alkaline. In such cases he thought  
the use of ammonium salts in dentifrices might be detrimental.

THE CHAIRMAN said that Dr. Kesel had recently stated that he  
hoped to be able to present statistically valid figures on  
the use of ammoniated dentifrices within the next six to  
eight months.

5. DR 2 - Dr. G.H.W. Lucas

"A study of alveolar oxygen tension and blood  
oxygen content during nitrous oxide-oxygen  
anaesthesia"

DR. LUCAS presented a summary of the work done on this  
project which appears in APPENDIX "S"





DR. LUCAS said that Dr. Tickle is now overseas and as a consequence work on the project has been discontinued temporarily. He hoped it might be possible to do further work on this subject and that he would be successful in finding someone to carry on the investigation from the point where Dr. Tickle had left the work. It would be of interest, he thought, to investigate the question of where the extra oxygen in the expired gas came from but the great difficulty was to obtain patients on whom the necessary oxygen studies could be carried out while they were under anaesthesia. He said he hoped to come back to the Committee for additional funds and he wondered whether this would be an opportune time to raise the question as to whether the Committee would support further work on this project.

It was MOVED by Dr. Charron and SECONDED by Dr. Collip:

That this Committee is in favour of receiving an application from Prof. Lucas for further work under project DR 2. CARRIED.

6. DR 3 - Dr. H. K. Box

"A study of micro-organisms found in periodontal pockets and dental caries as revealed by the electron microscope"

DR. BOX said that work was proceeding under this project whenever suitable material for study in the electron microscope became available.

7. DR 4 - Dr. H. K. Box

"Periodontal Packs" - Project completed.

8. DR 5 - Dr. R. L. Twible

"Denture base materials" - Project completed.

9. DR 6 - Col. E. M. Wansbrough

"To study the effects of altitude acceleration and deceleration on oral tissues"

COL. WANSBROUGH said that the officer who has been in charge of this investigation is being re-posted as of 1 November, 1949, and he hoped it would be possible to present a report on this investigation at the next Meeting of the Committee.

10. DR 7 - Dr. J. S. Bagnall

"Investigation and critical report on dental caries research"





THE CHAIRMAN said that Dr. Bagnall had now completed a monumental study on the literature relating to caries research of which the principal headings had been presented for the information of the Committee in Appendix "J" of the proceedings of the 9th Meeting. He said that Dr. Bagnall had now submitted a three-volume manuscript of 1175 pages to the Committee and the disposition of this manuscript would be dealt with later in the meeting. He then invited Dr. Bagnall to present a shorter manuscript and bibliography entitled "Trends in Caries Research" which appears in full in

APPENDIX "T"

DR. BAGNALL said that the summary to which the Chairman had referred contained a brief review of the work on caries which he thought might be of general interest to the dental profession. He said that in his opinion it would be profitable to support research on three lines of work dealt with in this study. These were (i) further investigation of the place of the ammonia ion; (ii) further study on the use of fluorides since it is not yet known why the incidence of caries is less in areas where fluorine is present either in the soil or in the communal water supplies; (iii) metabolic requirements in relation to teeth.

THE CHAIRMAN asked for suggestions as to the disposition of the summary and the complete report prepared by Dr. Bagnall. This was a piece of work that had been carried on over the last four years and the compilation represented a very important contribution to dental literature. It was important that the best possible use be made of it.

After the subject had been discussed at some length it was MOVED by Dr. Rae and SECONDED by Dr. McDougall,

That the Executive be asked to arrange for the distribution of the summary report on "Trends in caries research" including the selected bibliography, to all members of the dental profession in Canada, and

That the Executive also be asked to look into the problem of printing and distributing the complete report prepared by Dr. Bagnall on caries studies.

CARRIED.

11. DR 8 - Dr. A.D.A. Mason

"D.M.F. caries incidence in Brantford children" -  
Project completed.

THE CHAIRMAN said that the work initiated by Dr. Mason on the study of the teeth of Brantford children had been taken up by the Department of National Health and Welfare and the study had





been extended to include children from Sarnia where no fluorine is being added to the water and children from Stratford where about 1.3 p.p.m. fluorine occurs in the communal water supplies. The earlier work undertaken by Dr. Mason in Brantford was a rather hurried attempt to get a picture of the condition of the teeth of children in that city before any effects of the artificial addition of fluorine to the water supply had taken place. The study as it is now being carried on under Dr. Brown is much more comprehensive in nature.

DR. BROWN had asked for advice as to how frequently dental examinations of the children's teeth should be made. His own preference was for once in three years unless the committee advised him differently.

DR. BOX suggested that the study should be extended to the periodontal field at least in Stratford.

DR. GARVIN said that Gottlieb had recently stated that the addition of fluorine to water supplies meant that people living in such areas would lose their teeth ten years earlier than those who lived in communities where no fluorine is added to the drinking water supplies. He thought it would be useful to assess the situation among adults in Stratford where fluorine has always been present in the water supply.

DR. McDUGALL suggested that the three-year interval proposed by Dr. Brown was in line with accepted practice in regard to the topical application of fluorine to teeth and the Committee agreed that the three-year interval should be recommended to Dr. Brown.

12. DR 9 - Drs. O. J. Walker and H. R. MacLean

"An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry"

THE CHAIRMAN presented the summary of the work on this project which appears in APPENDIX "K"

THE CHAIRMAN said that the Committee's policy in regard to the publication of papers would be dealt with later in the meeting and the decisions then reached could be sent to Dr. Walker regarding the manuscript referred to in his summary. The manuscript was now in the hands of the Secretary of the Committee.

13. DR 10 - Dr. G. M. MacDonald

"Facial prostheses of vinyl resins" - Project completed.





14. DR 11 - Dr. R. J. Godfrey

"An anatomical, histological and physiological study of the role of the condyle in mastication" -  
Project completed.

THE CHAIRMAN reported that a paper on the work of this project had been published by Dr. C. H. Moses.

15. DR 12 - Dr. A. E. R. Westman

"Investigation of cements for methyl methacrylate inlays"

MR. D.R. CHRISTIE presented a summary of work on this project which appears in APPENDIX "F"

He pointed out that pending further work under DR 21 and DR 25 the study of cements is being held in abeyance.

16. DR 13 - Dr. M. Doreen Smith

"A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario"

THE CHAIRMAN presented a summary and an interim report prepared by Dr. M. Doreen Smith. These two documents appear in APPENDIX "Q"

DR. BAGNALL said that he wished to compliment Dr. Smith on her work on topical application which he thought was new. He said also that in his opinion the hardness of teeth could only be measured with accuracy over a long series of samples. Otherwise the differences observed in the hardness of individual teeth would be misleading.

THE CHAIRMAN read an extract from Chemical and Engineering News, 22 August, 1949, page 2410, which he said had been brought to the attention of Dr. Smith and might also be of interest to members of the Committee. The extract is as follows:

"The experimental use of fluorine in drinking water to prevent tooth decay is premature, says D.C. Badger, a physician of Hobbs, N.M. He says the use of one p.p.m. fluorine in this manner has not had the study that dental fluorosis has had, but the amount of fluorine which is non-toxic and also effective in tooth decay has not been established by all investigators. Stating that he is a practising physician in an area where water contains fluorine, he





reported that among children - 81 cases - examined in this area dental fluorosis was observed in 30% of the children who drank water containing 0.9 p.p.m. of fluorine. He recommends lowering municipal water supply content to 0.7 p.p.m. and giving distilled water to children up to six years of age (C and EN, June 13, page 1707)."

17. DR 14 - Dr. M. Doreen Smith

"Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries" -

Project completed.

18. DR 15 - Dr. Norma Ford Walker

"A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches"

THE CHAIRMAN presented an interim report by Dr. Walker which appears in

APPENDIX "G"

He said that it appeared that valuable results were being secured on the patterns of eruption of teeth in children.

DR. THOMSON said that apparently all the data had been collected on patterns of eruption but that no information had yet been presented on the main problem of arch width and eruption as in all probability the statistics on this phase of the work had not yet been compiled and interpreted.

THE CHAIRMAN said that he thought the actual measurement of arches had not yet been made and probably could not be made for a few years yet when the children were older; he thought however Dr. Walker might be asked regarding this point and that it might be suggested that she should include a brief note of explanation in her next report. He said that Henley had recently published a paper indicating that it might be possible to predict whether malocclusions would occur, if the pattern of eruption were known.

19. DR 16 - Dr. C. H. Best

"In vivo studies of certain fungus-like micro-organisms found in periodontal disease" -

Project completed.

20. DR 17 - Dr. R. F. Shaner

"An investigation of the effects of materials used in operative dentistry on the vital pulps of teeth" - Project discontinued pending the finding of a successor to Dr. Shaner to carry on the work.





21. DR 18 - Dr. H. K. Box

"Experimental artificial production of dental caries in vitro"

DR. BOX presented an interim report which appears in APPENDIX "N"

THE CHAIRMAN said that Dr. Hugill was making good progress on this study under the direction of Dr. Box and he suggested that the Committee might be interested in having Dr. Hugill come to the next Meeting to present a review of his work under this project.

22. DR 19 - Dr. J.K.W. Ferguson

"A comparison of local anaesthetics for conduction block"

THE CHAIRMAN presented Dr. Ferguson's summary of his work which appears in APPENDIX "D"

23. DR 20 - Dr. Jules Thébaud

"Improvement of sub-gingival curettage and gum resection techniques"

THE CHAIRMAN presented and read the report by Dr. Thébaud which appears in APPENDIX "R"

He noted that the periodontal micrometer which Dr. Thébaud wished to patent had been forwarded to the National Research Council in accordance with a request from Mr. A. C. Halferdahl, Officer-in-Charge of Patents. He said that there had been an exchange of correspondence on this subject and that apparently Dr. Thébaud felt that he was obliged by the regulations governing grants-in-aid to submit his new design of a micrometer to the Council for patenting. Actually Dr. Thébaud could have filed an application directly in the matter and notified the Council who would under the regulations have an interest in any patents thus secured. There was some doubt as to whether such a device as that developed by Dr. Thébaud could or should be patented but as the matter was being investigated by Mr. Halferdahl it did not appear that any further action needed to be taken at present by the Committee.

It was MOVED by Dr. Thomson and SECONDED by Dr. McDougall,

That this Committee subscribes to the view expressed by the Chairman in his letter of 27 May to the effect that "If it is a simple and inexpensive matter, perhaps there is little to be lost by proceeding according to Dr. Thébaud's wishes. On the other hand, if the process is involved and expensive, I would be quite opposed to proceeding further".

CARRIED.





24. DR 21 - Dr. A.E.R. Westman

"Investigation of repair techniques for methacrylate dentures"

MR. CHRISTIE presented a summary report, a supplementary report on DR 21, and the regular Ontario Research Foundation report No. 4903 on this subject, all of which documents appear in  
APPENDIX "A"

He said that the paper referred to in the summary has been accepted for publication and will appear in the November issue of the Journal of the Canadian Dental Association.

25. DR 22 - Dr. C. H. Best

"An investigation of the mechanism of repair of the supporting structures of the teeth"

DR. LINGHORNE presented a comprehensive report illustrated with lantern slides that occupied the attention of the Committee for the better part of an hour. A summary of the project appears in  
APPENDIX "C"

THE CHAIRMAN said that he had discussed this project with the grantee, Dr. Best, and had found him very enthusiastic over the work done so far. It appeared to be the consensus of opinion that excellent progress is being made and he wished to congratulate Dr. Linghorne on the success thus far achieved. He had also received a letter from Dr. Schour of the University of Illinois on this subject of which he read the following excerpt:

"I had an opportunity, as you know, to examine Dr. Linghorne's research study together with Doctors Weinmann and Sicher. We were fortunately able to give him a good portion of two days. We were impressed with his work, and feel that he should be encouraged and supported in his further progress.

"The problem of re-attachment of periodontal structures has been given considerable discussion and thought, but mostly on a theoretical basis. Doctor Linghorne's experimental approach appears to be the first sound attack which gives objective evidence of the possibilities of inducing re-attachment under special experimental conditions. We feel that his findings should be published in spite of the fact that his material would have been even more valuable if he had had celloidin imbedding of some of his material, and the assistance of a technician more experienced in the preparation of dental and periodontal tissues. We have written to Doctor Best, who, I am sure, will be glad to show you our letter."





## 26. Meeting of the Executive

THE CHAIRMAN announced that a Meeting of the Executive would be held during the evening at 8 P.M., in room 311, at the Chateau Laurier.

The First Session of the Meeting adjourned at 5 P.M., and the Second Session was convened at 9:30 A.M., Tuesday, 18 October 1949.

### SECOND SESSION 18 October, 1949

## 27. Attendance

Dr. R. G. Ellis, Chairman  
Dr. E. Charron  
Dr. M. H. Garvin  
Dr. R. H. McDougall  
Dr. J. J. Rae  
Dr. D. L. Thomson  
Col. E. M. Wansbrough

Mr. S. J. Cook, Secretary

### By Invitation

Dr. J. Stanley Bagnall  
Dr. L. S. Bélanger

### Reports of Grantees (cont'd)

## 28. DR 23 - Dr. C. P. Leblond

"Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon"

THE CHAIRMAN welcomed Dr. Bélanger who presented a detailed report with slides on the work done under project DR 23 of which a summary appears in

APPENDIX "E"

He said that he had read the paper submitted by Dr. Leblond which is mentioned in the summary and that he had returned it to Dr. Leblond for publication.

DR. THOMSON said that he had talked with Dr. Leblond regarding this project. The technique of contact autographs developed by Dr. Leblond was excellent. Dr. Thomson said he was enormously impressed with the originality of the work and he thought the outlook for success was very promising. He was sure the results of the investigation would provide a much clearer picture than has been had heretofore regarding the enamel.





THE CHAIRMAN said that work was being done at Toronto with a view to the production of secondary dentine rapidly in order to provide the extra protection to the pulp that was needed prior to removal of carious material from deep cavities. He believed the technique used by Dr. Leblond might prove valuable in this investigation into the deposition of secondary dentine and wondered whether this method could be used.

DR. BELANGER thought this would be possible provided that sufficiently thin sections could be secured.

29. DR 24 - Dr. C.H.M. Williams

"The effects of hard and soft diets on the supporting structures of the teeth"

THE CHAIRMAN presented an interim report by Dr. Williams and said that he had invited Dr. Williams to be present at this meeting but that both he and Dr. Watt had asked leave to attend a later meeting when their work would be further advanced. He said that he had seen a comprehensive report on the work done to date and had collected opinions to the effect that the results were very interesting and that it had been a good piece of work. The interim report by Dr. Williams appears in  
APPENDIX "M"

30. DR 25 - Dr. A.E.R. Westman

"Investigation of the use of methylmethacrylate for direct intra-oral fillings"

MR. CHRISTIE presented an interim report on this subject and Ontario Research Foundation report No. 4901 at the First Session of the Committee. These two documents appear in  
APPENDIX "B"

31. DR 26 - Dr. R. E. Moyers

"Measurement of physiological forces in the mouth"

THE CHAIRMAN stated that work had just been undertaken on this project and that a brief statement to this effect made by Dr. Moyers appears in  
APPENDIX "J"

32. DR 27 - Drs. J. W. Abbiss and A.G. Nutlay

"A histological study of tooth germ development both intra- and extra- alveolar with a survey of pathological changes in the developing tooth"

THE CHAIRMAN submitted a summary of work on this project prepared by the grantees. It appears in  
APPENDIX "H"





He pointed out that in accordance with the note concerning the grant for this project which appeared in minute 31 page 13 of the proceedings of the 9th Meeting, the Executive had corresponded with the grantees regarding their laboratory equipment and the actual need for the purchase of a sledge microtome. A mail ballot of the Executive had then been taken which resulted in the grant being approved which had been encumbered at the 9th Meeting. He thought it would be useful to have either Dr. Abbiss, Head of the Department of Pathology or Dr. Nutlay, who is doing the work under this grant, present at the next Meeting of the Committee to report in person. A paper on the work done under this project has been published. He felt sure that the results of this research would provide valuable material both for teaching and for further scientific studies.

33. DR 28 - Dr. O. J. Walker

"Determination of small amounts of fluorine by means of the photoelectric colorimeter"

A summary of the work done by Dr. Walker  
appears in APPENDIX "L"

34. DR 29 - Dr. M. Doreen Smith

"Biological absorption of fluorine"

A summary and an interim report on DR 29  
appear in APPENDIX "P"

35. DR 30 - Dr. Norma Ford Walker

"Study of closure of space following premature loss of deciduous teeth"

An interim report on this project appears  
in APPENDIX "I"

DR. GARVIN said that the work being done by Dr. Norma Ford Walker was likely to prove a valuable guide to practising dentists. At present knowledge is limited as to the need or not, of placing devices in spaces to keep the space open for the eruption of secondary teeth. In some cases such devices are required and in other instances they are quite unnecessary. The usual practise at the present time, following premature extraction, is to observe the cavity at regular intervals but this is not a very satisfactory procedure.

DR. BAGNALL said that Dr. Mainland is also doing work on skeleton growth and he thought that the work being done by Dr. Walker should be correlated with Dr. Mainland's research.







DR. CHARRON said that data on growth would be extremely useful to the Committee as a guide to the validity of research projects put forward for its attention. He thought that a paper indicating the present state of knowledge in this field and the directions in which research is proceeding would be of great value. He suggested that consideration be given to this matter and that if possible a competent authority on the subject be invited to address the Committee at its next Meeting.

DR. THOMSON agreed that Dr. Charron had made an excellent suggestion and he proposed that Dr. Mainland be invited to make the presentation. 11

THE CHAIRMAN thanked Dr. Thomson and Dr. Charron for their suggestions and said that he thought it would be very useful indeed to have such an item on the programme for a future meeting.

### 36. Method of Tooth Repair

THE CHAIRMAN said that considerable publicity had been given in the press recently to an experiment made by Dr. D. F. Stedman, of the Division of Chemistry, National Research Council. It appeared that Dr. Stedman had succeeded in filling a cavity in one of his own teeth by means of a paste consisting of ingredients similar to the composition of a tooth. This project had not been considered by the Associate Committee on Dental Research but many who had read the item in the papers supposed that it was one of the Committee's projects and this had led to some embarrassment.

DR. MACKENZIE said that Dr. Stedman had unusual ability as a chemist and that he had many ideas which were not always considered orthodox by other scientists but despite this fact he had made many excellent contributions to the work of the Council and any suggestions from him could not be dismissed lightly.

COL. WANSBROUGH said that Dr. Stedman had told him of the results achieved on the single tooth and had suggested that the method be tried on a wider scale to ascertain whether it had any value. Col. Wansbrough said that he thought it was worth trying and he had accordingly offered the services of the Royal Canadian Dental Corps in this connection. Many volunteers are having cavities treated at the present time. The method is rather tedious in that the applications of the remedy have to be replaced nearly every day and some of the patients have grown tired of the experiment. He had no results to report as yet but he felt that he had been warranted in undertaking the trial.





DR. CHARRON thought there was no need for the Committee to take any action in the matter as the Dental Corps was conducting a study and would no doubt inform the Committee if useful results were obtained.

DR. BOX said that he had filled cavities this way for several years but he thought it should be pointed out that the procedure did not result in the restoration of normal enamel but produced a calculus-like substance which was neither dentine nor enamel but might constitute a satisfactory filling in certain cases.

DR. BAGNALL suggested that Col. Wansbrough keep a record of the calculus formation in the test cases that were being tried.

Other members expressed the view that the experiment being undertaken by the Dental Corps was quite worthwhile.

37. Report of Subcommittee on Publications(Min.35, 9th Mtg.)

THE CHAIRMAN drew attention to the fact that the report of the Subcommittee on Publications presented at the 9th Meeting had been included in the proceedings of that Meeting for consideration by the members and that the necessary action thereon was to be taken at this meeting. Copies of the report of the Subcommittee were distributed to the members and the following action was taken:

- (i) It was MOVED by Dr. Rae and SECONDED by Dr. Thomson

That para. 2(v) of the report be amended to read as follows:

"The grantee shall supply, when they become available, twenty-five reprints of each publication for the use of and at the expense of the National Research Council".

- (ii) It was MOVED by Dr. Rae and SECONDED by Dr. Lucas,

That para.2(vi) be amended to read as follows:

"Reprints of each publication shall be available to each member of the Associate Committee on Dental Research ".

- (iii) It was MOVED by Dr. Charron and SECONDED by Dr. McDougall,

That the report of the Subcommittee on Publications as published in Appendix "S", 9th Meeting, be approved as amended. CARRIED.





The procedure in regard to publication  
as thus approved appears in APPENDIX "V"

In view of the foregoing decision regarding the stock of reprints to be maintained in the National Research Council's offices, the Secretary inquired what should be done with the very considerable number of reprints of various dental papers he has on hand at the present time.

It was AGREED that the stock of reprints of dental papers in the hands of the Secretary should be reduced to not more than twenty-five copies of each reprint and that any surplus over this number should be returned to the authors.

### 38. Membership of the Committee

THE CHAIRMAN reported that the National Research Council had approved the reappointment of four members of the Committee for a further term of three years to 31 March, 1952. These members are: Dr. H. K. Box, Dr. J. B. Collip, Dr. A.W. Ham, and Dr. R. H. McDougall.

THE CHAIRMAN said that all members of the Committee were gratified that these appointments had been renewed and that the Committee would consequently continue to have the benefit of the advice of these members whose assistance in the past had been so helpful.

### 39. Finances

THE CHAIRMAN reported that it had been necessary to make a tentative estimate of the Committee's financial needs in September 1949 and it had been decided, subject to approval of the Committee, to recommend the same appropriation as for 1949-50. These requirements are as follows:

|               |                 |
|---------------|-----------------|
| Grants-in-aid | \$27,500        |
| Fellowships   | 5,000           |
| Travel        | 2,500           |
| Contingencies | 500             |
| Total         | <u>\$35,500</u> |

It was MOVED by Dr. Thomson and SECONDED  
by Col. Wansbrough,

That the tentative estimates for the  
Committee's requirements in 1950-51 in the amount  
of \$35,500. as detailed above be approved. CARRIED.

### 40. Fellowships (Min. 33, 9th Mtg.)

THE CHAIRMAN said that the National Research Council had amended its regulations regarding grants and fellowships and in response to a request from the General Secretary of the Council he had agreed that the dental fellowship forms should be revised to make them conform to the other fellowship forms







issued by the Council. The amendments made provide (i) that a Fellow shall submit a report on his work on the conclusion of the tenure of his Fellowship or not later than 15 September following this conclusion and in the case where a Fellowship is renewed, at the end of the first year of tenure; (ii) that on awards tenable in Canada fifty percent of the grant will be paid on notification from the supervisor that a Fellow has reported for work, forty percent on receipt of a report from the supervisor that satisfactory progress has been made, and ten percent on completion of the period of tenure and submission to the Council of a satisfactory report; (iii) that on awards tenable outside Canada payment of fifty percent shall be made not more than one month prior to the departure of the grantee and fifty percent at the mid-term following report from the supervisor; (iv) provision is also made for a travelling grant on the basis of single coach railway fare less \$15; and for travel to Europe, \$350.

It was AGREED that the foregoing revisions be APPROVED.

The revised forms are now being printed and will be distributed in the same way as they were last year namely to the Deans of Dental Schools, the Canadian Dental Association, dental journals, Royal Canadian Dental Corps, Provincial Dental Licensing Board, Registrars of Canadian universities and members of the Committee.

#### 41. Air Travel (Min. 5, 9th Mtg.)

THE CHAIRMAN reported that a small item regarding air travel needed to be dealt with as a supplement to the provision made in the proceedings of the 9th Meeting. He said that Dr. W. J. Linghorne had travelled to Ottawa at the invitation of the Committee and that he had both come and returned by air for which travel authorization by the Committee was required.

THE CHAIRMAN said also that he himself found it necessary to return to Toronto by air as he had to keep a speaking engagement in Toronto tonight.

It was MOVED by Dr. Garvin and SECONDED by Col. Wansbrough,

That travel by air be authorized for Dr. Linghorne's trip Toronto to Ottawa and return and for the Chairman of the Committee Ottawa to Toronto on this date and also as may be required at any other time during the present fiscal year.

CARRIED.

The Meeting adjourned at 12:10 P.M.







42. Attendance of Grantees at Meetings of the Committee

THE CHAIRMAN said that it had been found very useful to have grantees or their assistants present at meetings of the Committee to report personally on their projects. He thought it would be of interest to the Committee to know how this plan had been employed and he had therefore made a compilation showing by meetings the names of the grantees and the persons reporting on various projects. This compilation appears in

APPENDIX "U"

In the light of the discussion at the present meeting it appeared desirable to invite a number of workers to attend the next meeting of the Committee. Names suggested to him included - Dr. Mainland of Dalhousie University; Dr. Norma Ford Walker (DR 15 and DR 30); Dr. M. Doreen Smith (DR 13 and DR 29); Dr. C.H.M. Williams or Dr. D.G. Watt (DR 24); Dr. R.L. Hugill (DR 18); Dr. J.W. Abbiss or A.G. Nutlay (DR 27).

DR. CHARRON said that he would also like to have the situation regarding Dr. Thébaud's grant (DR 20) cleared up at the next meeting of the Committee.

43. Report of the Executive

THE CHAIRMAN stated that the 7th Meeting of the Executive had been held in room 311, Chateau Laurier, 8 to 10 P.M., 17 October, and the proceedings had been prepared. He asked the Secretary to read the Minutes of the Executive Meeting. (The proceedings of the Executive have been distributed to the members of the Committee).

THE CHAIRMAN said that the proceedings were open for discussion and subject to amendment particularly because two of the members of the Executive who were unable to be present at the Executive Meeting were present at this Session of the Committee. A few minor amendments were suggested and these were incorporated in the proceedings of the Executive before they were distributed.

It was MOVED by Dr. Garvin and SECONDED by Dr. McDougall,

That the proceedings of the 7th Meeting of the Executive be received and APPROVED. CARRIED.

44. Date of Next Meeting

It was AGREED that the next meeting of the Committee should be held in February 1950 at the call of the Chair.

The Meeting adjourned at 12:10 P.M.





## APPENDIX "A"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: Improved methods for repairing methacrylate dentures.

Grantee: Dr. A. E. R. Westman

Project

No.: DR 21      Amount of Grant: \$4,000 (including DR 12  
and DR 25)

#### SUMMARY OF WORK

As this investigation developed it became apparent that there are two phases to the problem requiring somewhat different experimental treatment. The first phase dealing with the repair of fractures and the replacement of teeth was finally reported upon in Report 4902, dated March 1, 1949. The second phase, dealing with the re-lining of dentures, has been partially reported upon in progress reports dated May 5th and July 29th.

A paper has been prepared for publication on the first phase, and this is now in the hands of the publishers.

In the work on the second phase, it has been found that when the new lining material contracts during the curing process, it draws the old material with it, thus distorting the whole denture. Experiments of a preliminary type indicate that the solution is to provide for a lining material that will remain relatively softer than the denture when finally cured. The problem is being attacked in two ways, a) by modifying the cure with an inhibitor and b) by the use of a modified acrylic resin which is internally plasticized.

September 23, 1949.

O. J. Schierholtz  
for Dr. Westman





## APPENDIX "A"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Ontario Research Foundation  
Department of Chemistry

Dental Materials Research Fellowship

Supplementary Report No. 1 - DR 21

#### Scope of This Report

Additional studies of the problems involved in the distortion of lower dentures on relining are described in this report. A solution to this problem is proposed and some of the preliminary experiments which have been started in this regard are outlined.

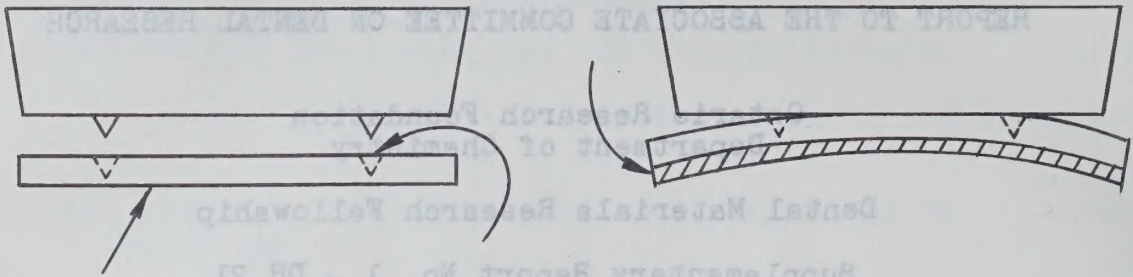
#### Cause of Distortion on Relining

Although a summary report on project DR-21 was filed for the last meeting of The Associate Committee on Dental Research, a satisfactory solution to the important problem of relining distortion had not been obtained. It was considered highly desirable to investigate this matter more fully in the hope of providing a complete answer to the general problem of repair distortion. Our latest findings point the way to success.

We have studied the effects produced by adding new denture base material, i.e. "relining", to flat strips of fully cured methyl methacrylate which were also thoroughly conditioned in water. The strips measured approximately 1/8 X 1 X 3 inches, and an index of one of the original surfaces was made. It was found that the addition of new material to one side of the strips caused a bowing of the whole strip, such that the original reference surface became convex in shape.



The following simple diagram illustrates this point:-



This result occurred no matter whether the reline was carried out at room temperature or at 212°F and indicates that the distortion is caused by the strong forces set up in the new material as it contracts on polymerization. This same effect is apparent in lower dentures which are relined when it is found that the denture "pinches-in" at the heels and will no longer fit its model or occlusal index.

#### Methods of Eliminating Reline Distortion

There are two methods which may prove effective in eliminating reline distortion. Both have as their objective the production of a soft resin which, by reason of its flexibility, will not distort the original material. First, it may be possible to add an inhibitor or retarder to the reline material which will produce a low molecular weight polymer with some degree of resiliency and which will remain in this flexible condition. The second method would be to use a resilient or flexible resin in the reline material. We do not propose the use of a plasticized resin containing a plasticizer which can be leached out in time but rather a soft acrylic resin, such as butyl methacrylate, which would give permanent internal plasticization.

All cures should be carried out at 135° F to avoid distortion by the release of strains.

#### Retarders or Inhibitors

We have made some experiments with hydroquinone as the retarding agent. The hydroquinone content of monomeric methyl methacrylate was 0.25% and was used in the usual manner with the polymer powder. Since a very soft resin is produced, it seemed advisable to provide a hard external surface on the added material which would protect the soft internal layer.



This was done by trial-packing the new resin in the ordinary way and, when the trial-packing was judged to be adequate, the exposed surface was painted with monomer containing 3% trihexylamine and sprinkled with polymer powder. This procedure was repeated after a trial-closure of the flask and then curing was carried out in a water-bath at 135° F.

First indications of this method were very promising as no evidence of distortion of the flat strips was present. However, 10 days to two weeks after the "reline," the strips began to warp, and in a few days were poor fits on their indices. A yellow discoloration of the resin containing excess hydroquinone was also observed. This indicated oxidation of the hydroquinone with the resultant loss of its inhibiting powers. The added resin then continued to polymerize and in doing so its shrinkage distorted the flat test specimen.

This method has been continued with the use of a more stable retarder, 2,5 ditertiary butyl hydroquinone. At this writing, specimens "relined" two weeks ago are still free of distortion.

### Internal Plasticization

Permanent flexibility of the added resin in relines can be assured by the use of a soft acrylic resin, either as the monomeric component, as part of monomeric component or used as the polymer in liquid-powder mixtures.

We have made some initial investigations with materials on hand but have not been able to produce a polymer which is flexible enough to prevent distortion of the original denture base material. Those combinations which were not successful were:

- (1) ethyl methacrylate monomer + Lucitone polymer
- (2) 20% solution of the polymer from Acrysol C-9 in methyl methacrylate monomer + Lucitone polymer
- (3) 20% solution of the polymer from Acrysol ER in methyl methacrylate monomer + Lucitone polymer
- (4) 20% solution of the polymer from Acrysol ER in ethyl methacrylate monomer + Lucitone polymer.

We are awaiting the arrival of several soft acrylic resins which may prove more useful than those already tried.







## APPENDIX "A"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Ontario Research Foundation  
Department of Chemistry

Dental Materials Research Fellowship

Report No. 4903 - DR 21 \*

#### Scope of this Report

Since the last local committee meeting the work on this project has been devoted to a study of the relining of lower dentures. An outline of this work is described in this report.

#### Osborne's Technique

At the last meeting our attention was drawn to a method of repairing dentures which appeared in the British Dental Journal under the name of John Osborne. It was considered desirable to investigate the claims put forth in this paper.

In brief, Osborne's method is an attempt to avoid distortion caused by repairing at relatively high curing temperatures and at the same time to cure the new denture base material in a convenient time. His technique is to heat the invisted denture at 212° F for exactly 10 minutes and then allow the dental flask to bench cool to approximately room temperature. In the time required for the flask to cool, the added denture base material is polymerized to a satisfactory degree.

Osborne states that the denture never attains a temperature higher than 170° F and that the above-mentioned technique has proved very satisfactory in curing many clinical cases.

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\* This report represents some work which was done to round out the final report on DR 21 published as Appendix "B" of the 9th Meeting of the Associate Committee on Dental Research.



We find that his technique, insofar as we can interpret it from the publication, does not avoid distortion and in fact, by our standards, the fit of the dentures on models and occlusal indices is poor. Relines and repairs were investigated by this method and found to be rather inferior.

### Ultra-Violet Method

Previous experiments on relining by the ultra-violet method revealed that considerable distortion occurred. It was decided to attempt to increase the degree of polymerization of the added denture base material during the preliminary heat treatment at 135° F, so that when irradiation was carried out polymerization shrinkage would be reduced.

To accomplish this, trihexylamine in a concentration of 3% in the monomer was used. The preliminary heat treatment was maintained at 2 hours and irradiation for 1½ hours.

Only one case was processed in this manner when it was decided to abandon this technique altogether for relines. Although this particular trial was fairly successful and only a slight distortion occurred, the difficulties encountered in opening the flask after the preliminary heat treatment were considered to be a serious drawback and time-consuming obstacle. Despite the fact that the denture possessed absolutely no undercuts on the tissue side, the cast broke off and almost the entire ridge remained in the denture. This result was not due to a poor separating medium but entirely to mechanical retention of the artificial stone. In cases where the ridge was undercut, flask separation would be just as difficult or next to impossible.

Relining, then, is not feasible by a method which requires flask separation such as the ultra-violet and infra-red methods which can be conveniently applied to other types of repairs. A successful relining method will have to be carried to completion with the denture kept in a closed flask.

### Trihexylamine at 135°F

Another method of bringing the added resin to a higher degree of polymerization than would be produced by low-temperature curing alone, is to incorporate a catalyst which is active at a low temperature. Trihexylamine (THA) in conjunction with benzoyl peroxide is an effective catalyst.

Previous data reveal that Lucitone denture base material attains a Knoop hardness value of 13 to 13.5 after curing at 135° F for 16 hours. With 3% THA in the monomer, a mixture attains a hardness of 12.4 in 3½ hours and 15.0 in 16 hours at 135°F.



Accordingly, lower dentures were relined at 135° F for 3½ hours using THA in the mixture of monomer and polymer. The results obtained were as good as by any method yet tried. A slight distortion occurred, however. It is not known whether this change in dimensions is serious enough to affect the serviceability of the denture.

The reason for the distortion is not readily apparent. When other types of repairs are made under approximately the same conditions no distortion occurs. This indicates that where the bulk of original denture base is sufficient to resist any dimensional change, the curing conditions do not cause warpage of the denture by releasing strains. In a re-line, the added material seems to exert enough tension, probably because of polymerization shrinkage alone, to distort the denture by pinching in the heels.

In another approach to this problem, flat strips of denture base material were cured by heating from room temperature to 212°F in about one hour, conditioned in water for a day and then "relined" by having new material added to one side. The relining conditions were as above and the thicknesses of the new material in the finished "relined" strips were 25 and 40 per cent. In these experiments, a distortion of the original flat strips occurred in a manner which suggests that curing shrinkage of the new material is the causative factor: the new surface became concave in shape while a corresponding convexity developed on the strip previously cured at 212°F.

It was hoped that curing shrinkage could be overcome to some extent if a larger volume of a very fine polymer powder could be mixed with a given volume of monomer. A polymer of 250-mesh and finer particles was obtained by sieving a clear sample of Kallodentine denture base material. However, it was found that the use of this polymer did not overcome the distortion of flat strips, for even though a larger amount of polymer seemed to be incorporated in the monomer the actual weight was less. A check showed that the fraction of voids in Lucitone pink polymer was 0.42 while the fraction in the fine polymer was 0.52. This difference is caused by the variation in the particle sizes of Lucitone which gives better packing and the relative lack of variation in particle sizes in the sieved polymer. Furthermore, it was found that in a mixture of workable consistency, 2.85 grams of Lucitone could be mixed with one cc. of monomer while only 2.42 grams of the fine polymer could be used with one cc. of monomer.

Some additional experiments are being carried out with the object of obtaining more information on this problem of distortion of relines.





APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: Investigation of the use of methylmethacrylate for direct intra-oral fillings.

Grantee: Dr. A. E. R. Westman

Project

No.: DR 25 Amount of Grant: \$4,000 (including DR 12 and DR 21)

SUMMARY OF WORK

Report 4901, dated July 29th, 1949, described the commencement of this experiment in which the effect of direct acrylic fillings on the pulp of vital teeth is being studied, using dogs as the experimental animals.

Since then, the dogs have been sacrificed, and the teeth are now being prepared for histopathological study.

The results of this experiment will be reported in due course.

September 23, 1949.

O.J. Schierholtz  
for Dr. Westman





## APPENDIX "B"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Ontario Research Foundation

Department of Chemistry

Dental Materials Research Fellowship

Report No. 4901 - DR 25

#### Scope of this Report

This report describes the start of an experiment to determine the affect of direct acrylic fillings on the pulp of vital teeth and to assess the suitability of a technique designed to cure these acrylic fillings.

#### Experimental Details

The subjects on which this experiment was carried out were two young dogs. One was estimated as about 5-6 months old and the other about one year old. The twelve anterior teeth in each dog were used and the experiment was designed by our Department of Mathematical Statistics to compare the affect on vital teeth of silicate and direct acrylic fillings with and without linings of zinc phosphate cement. The experimental design will be given in detail in a later report.

All cavities were of the Class V type and were prepared with retention undercuts. The direct acrylic fillings were made with inhibitor-free monomer containing 3% trihexylamine and Hue-lon acrylic inlay powder. Polymerization of the acrylic fillings in the cavities was effected by the use of a specially designed heat lamp. It is planned to sacrifice the animals after 7-8 weeks, which will be about September 1, and then make sections of the teeth and a histopathological study for pulp reaction.

It is considered that the technique of polymerizing the fillings will prove quite satisfactory when applied to individual human teeth. Some difficulty was encountered with the first two or three fillings which were inserted in the dogs but after a little experience the method was easily carried out.



"B-2"

The monomer-polymer mixture has to be used very soon, within a minute, after being mixed. Otherwise, it reaches the doughy stage and is difficult to handle. The addition of a slight amount of monomer will restore it to a workable consistency. Pressure must be applied immediately, preferably by a mold index or celluloid strip. In this experiment, the resin was compressed by finger pressure until the cavity was filled although this method fails to produce the contours necessary for satisfactory aesthetic fillings.

Curing was achieved by irradiating the acrylic filling with radiant heat energy through a quartz rod about 5 millimetres in diameter. It was found that a straight tapered rod would have been more effective in localizing the heat than the untapered rod which was used. An exposure of 15-20 seconds on the filling, followed by an interval of 15-20 seconds of no exposure to allow the tooth to cool proved satisfactory to harden the filling when repeated 5 or 6 times. The fillings were trimmed with sandpaper discs and polished with whiting and water. Their workability in these operations was considered very good.

The heat lamp which consists essentially of the illuminating elements of a movie projector, i.e., 125-watt lamp, reflector and condensing lens, worked well in this experiment but will have to be redesigned for practical use to provide more adequate cooling. The last filling had just been completed when the heat developed in the apparatus melted the glass post supporting the filament of the lamp, causing it to fall sideways and to stop operating.

The results of this experiment will be reported when the teeth have been examined.

July 29, 1949

Douglas R. Christie

## APPENDIX "C"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1949

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth.

Grantee: Dr. C. H. Best

Project: DR 22                      Amount of Grant: \$2,200.

#### SUMMARY OF WORK

Studies on the capacity for regeneration and reattachment of the supporting structures of the teeth and the factors and principles underlying their repair, have been carried out.

This report deals with the regeneration of the periodontal membrane and its reattachment to the tooth following the creation by surgical means of artificial pockets in dogs.

Studies of the repair process were made by means of histologic sections of specimens taken at healing intervals ranging from 1 day to 2 years.

September 20, 1949.

W. J. Linghorne, D.D.S.,  
Research Associate,  
Dept. of Physiology.

Date: Sept. 20, 1949

J. F. H. Ferguson  
Signature





APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1949.

Subject: A comparison of local anaesthetics for conduction block.

Grantee: J.K.W. Ferguson

Project: DR 19 Amount of Grant: \$350.

SUMMARY OF WORK

Injection behind the eyeball of the guinea pig has proved to be an excellent method of producing experimental conduction block, providing two ways of observing anaesthesia, namely dilatation of the pupil, and loss of the corneal reflex. The relative potency of various anaesthetics is somewhat different by this method as compared with intra-dermal injection. Forty minutes of anaesthesia is produced by 1% procaine on intra-dermal, or retro-bulbar injection. With nupercaine, however, 40 minutes of anaesthesia is produced by 0.01% on retro-bulbar injection, but by 0.0028% intra-dermally. Nupercaine shows in these tests as having the greatest potency and greatest factor of safety as regards tissue toxicity. Xylocaine, a new anaesthetic, has been disappointing in these tests.

Date: Sept. 20, 1949

J.K.W. Ferguson  
Signature





APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: Entry of phosphate and carbonate salts into teeth  
as shown with the help of radio-phosphorus and  
radio-carbon.

Grantee: Dr. C. P. Leblond

Project

No.: DR 23

Amount of Grant: \$2,200.

SUMMARY OF WORK

A copy of a paper, which has just been completed and is ready for publication, has been forwarded to Dr. Ellis.

It may be seen from this paper that the present results shed a new light on the process of formation of the diaphysis of long bones. However, while on the problem of bone formation the results could be interpreted with accuracy, those obtained on the problem of tooth development were more difficult to understand and it was therefore decided to withdraw them from this paper. Further experimentation is necessary to understand the results we have had with the teeth so far, especially the entry of radio-phosphorus in the dentin within five minutes after injection. Furthermore, some of our observations indicate that the mineral salts of enamel might come through the dentin, while the organic framework of enamel would be secreted by the ameloblasts. These results are, however, preliminary and need further confirmation.

Date: Sept. 17, 1949

C. P. Leblond  
\_\_\_\_\_  
Signature





## APPENDIX "F"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: Investigation of cements for methacrylate inlays

Grantee: Dr. A. E. R. Westman

Project

No.: DR 12

Amount of Grant: \$4,000

(including DR 21 and DR 25)

#### SUMMARY OF WORK

This project is being held in abeyance pending the outcome of DR 25 (Investigation of the Use of Methylmethacrylate for Direct Intra-Oral Fillings) since this may have a profound effect on the utilization of inlays in the future.

For the time being, we propose to confine our experimental work to DR 21 and DR 25, but will keep the literature on cements in view for possible important new developments.

September 23, 1949.

O.J. Schierholtz  
for Dr. Westman





## APPENDIX "G"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

**Subject:** A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches

**Grantee:** Dr. Norma Ford Walker

**Project No.** DR 15      **Amount of Grant:** \$2,500.00

#### INTERIM REPORT

The considerable body of data which was in the process of being collected for this project, was mentioned in the report submitted to the Committee in March 1949. In May of this year, the "books were closed", and final calculations were made for the mean eruption time for each deciduous tooth, separately and as homologous pairs, for sexes separated. The eruption times of the teeth as well as the widths of the dental arches are basic to this study. The first of these two requirements is now completed.

With a view to obtaining a reliable series, our files were completely revised, and a total of 360 records of eruption were selected. These were chosen on grounds of accuracy and reliability. The cases include:-

|              |             |       |   |       |       |                          |
|--------------|-------------|-------|---|-------|-------|--------------------------|
| Singletons   |             | 52 ♂♂ | : | 46 ♀♀ | Total | 98                       |
| Sibs         |             | 24 ♂♂ | : | 28 ♀♀ | "     | 52                       |
| <u>TWINS</u> | Monozygotic | 13 ♂♂ | : | 20 ♀♀ | "     | 33 pairs 66 individuals. |
|              | Dizygotic   | 19 ♂♂ | : | 13 ♀♀ | "     | 72 pairs 144 individuals |

Grand Total -

360 individuals.



From some two dozen published papers on the eruption of the deciduous teeth, one by Robinow, Richards and Anderson (1942) from the Fels Institute, was chosen for comparative purposes. The children in the Fels study, like the Toronto group, were "presumably normal healthy white children... from families of average or better than average economic status..." The Fels report was based on complete records of 64 children, incomplete records of 175 children, a total of 239. The Toronto report has complete records of 156 children, incomplete 204 children, a total of 360.

The order of eruption of the deciduous teeth for both boys and girls obtained in the Toronto study is in agreement with other writers, including Boas (1927), Sandler (1944) and Robinow, Richards and Anderson; this order being, lower centrals, upper centrals; upper laterals, lower laterals; first molars, upper or lower; cuspids, upper or lower; lower second molars and finally the upper second molars. There is, however, much evidence that variations from this order occur and it is with such variations that the present study is particularly concerned, especially with any association which these variations may have with the malocclusion series.

While the order of eruption for both boys and girls is in general agreement in both the Fels and Toronto studies, there are nevertheless some interesting differences. The Fels report indicates that throughout the entire eruption the boys are ahead of the girls, except in the eruption of the upper and lower first molars, in which case the girls are more precocious. The Toronto datum reveals that while the boys tend to be early in their upper centrals and laterals, as well as their cuspids, they are the same as the girls in the eruption of their first teeth, viz., the lower centrals, and that they are later than the girls not only in their lower laterals, upper and lower first molars (mentioned above), but also in the eruption of their upper and lower second molars. The only difference proven to be significant in the Toronto datum is the eruption of the upper second molars.

$$((S.E_1)_2 + (S.E_2)_2 = 14.297; 14.297 \times 2 = 28.594; \text{actual diff. of mean} = 33.94)$$

Meredith (1946) reviewing twenty-two papers on the eruption of the deciduous teeth concludes that "at all ages from nine months to two years, teeth tend to erupt earlier in males than in females".



"G-3"

Whether the inclusion of much data on twins has been responsible for the differences here shown between the sexes, is a point to be investigated. In twin births there is known to be a disturbance in foetal growth as shown by the increased frequency of miscarriages, neonatal deaths and physical anomalies. Has this disturbance of growth affected the development of the deciduous dentition of the multiple sets?

#### BIBLIOGRAPHY

- |                                 |   |      |                                                                                         |
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| Robinson, Richards and Anderson | - | 1942 | The eruption of the deciduous teeth. Growth. 6: 127-133.                                |
| Sandler, H.C.-                  |   | 1944 | The eruption of the deciduous teeth. J. Pediat., 25: 140-147.                           |

September 27, 1949

(Submitted by Margaret(Phillipotts-Brown) Hatton)

Norma Ford Walker  
Signature



"S-4"

IN DAYS  
ERUPTION TIME/OF THE TWENTY DECIDUOUS TEETH, taken SEPARATELY and in HOMOLOGOUS pairs.

A Comparison of the Toronto and Fels data. SEXES SEPARATED in both cases.

| REPORTS                                           |            | TORONTO                     |        |        |        | FELS INSTITUTE.                   |        |        |        |                                        |      |       |      |
|---------------------------------------------------|------------|-----------------------------|--------|--------|--------|-----------------------------------|--------|--------|--------|----------------------------------------|------|-------|------|
| Number with complete records                      |            | 156 children 87 ♀♀ ; 69 ♂♂  |        |        |        | 64 children. 33♀♀ ; 31 ♂♂         |        |        |        |                                        |      |       |      |
| Incomplete                                        |            | 204 children 94 ♀♀ ; 110 ♂♂ |        |        |        | 175 children                      |        |        |        |                                        |      |       |      |
| TOTAL.                                            |            | 360 children tooth          |        |        |        | 239 children.                     |        |        |        |                                        |      |       |      |
| Mean measure of <u>each</u> /of HOMOLOGOUS PAIRS. |            |                             |        |        |        | Mean measure of homologous pairs. |        |        |        | Mean Measure of homologous pairs only. |      |       |      |
|                                                   |            | ♀♀                          | s.d.   | ♂♂     | s.d.   | ♀♀                                | s.d.   | ♂♂     | s.d.   | ♀♀                                     | s.d. | ♂♂    | s.d. |
| L.C.I.                                            | L.CENTRALS | 232.29                      | 56.61  | 232.99 | 51.52  | 231.97                            | 56.26  | 231.99 | 50.49  | 234                                    | 63   | 219   | 48   |
| L.R.C.I.                                          |            | 231.66                      | 55.91  | 231.10 | 49.47  |                                   |        |        |        |                                        |      |       |      |
| U.C.I.                                            | U.CENTRALS | 286.69                      | 55.77  | 275.76 | 56.06  | 288.33                            | 56.88  | 279.60 | 55.04  | 288                                    | 60   | 273   | 45   |
| U.R.C.I.                                          |            | 287.98                      | 55.99  | 283.45 | 54.02  |                                   |        |        |        |                                        |      |       |      |
| U.L.L.I.                                          | U.LATERALS | 320.68                      | 69.48  | 309.56 | 59.56  | 321.79                            | 72.18  | 310.25 | 56.14  | 357                                    | 81   | 313.5 | 72   |
| U.R.L.I.                                          |            | 322.90                      | 74.84  | 310.94 | 52.78  |                                   |        |        |        |                                        |      |       |      |
| L.L.L.I.                                          | L.LATERALS | 373.60                      | 83.95  | 370.24 | 90.10  | 371.17                            | 84.79  | 373.62 | 86.54  | 414                                    | 118  | 390   | 84   |
| L.R.L.I.                                          |            | 373.65                      | 85.64  | 372.05 | 86.99  |                                   |        |        |        |                                        |      |       |      |
| L.F.M.                                            | L.F.MOLARS | 490.44                      | 96.24  | 495.00 | 92.81  | 480.74                            | 95.84  | 489.27 | 95.63  | 468                                    | 66   | 486   | 57   |
| L.R.M.                                            |            | 481.05                      | 95.45  | 483.55 | 78.45  |                                   |        |        |        |                                        |      |       |      |
| U.L.F.M.                                          | U.F.MOLARS | 477.65                      | 78.61  | 466.03 | 74.62  | 478.95                            | 79.04  | 486.95 | 74.87  | 471                                    | 69   | 480   | 69   |
| U.R.F.M.                                          |            | 480.26                      | 79.41  | 487.87 | 75.12  |                                   |        |        |        |                                        |      |       |      |
| U.C.                                              | U.CUSPIDS  | 579.38                      | 95.01  | 567.82 | 89.30  | 582.19                            | 94.37  | 569.22 | 90.48  | 603                                    | 96   | 567   | 81   |
| U.R.C.                                            |            | 585.00                      | 93.73  | 571.62 | 91.66  |                                   |        |        |        |                                        |      |       |      |
| L.C.                                              | L.CUSPIDS  | 560.18                      | 97.98  | 551.66 | 86.92  | 582.83                            | 96.66  | 582.66 | 90.64  | 606                                    | 112  | 579   | 81   |
| L.R.C.                                            |            | 585.49                      | 95.35  | 583.67 | 94.36  |                                   |        |        |        |                                        |      |       |      |
| L.L.S.M.                                          | L.S.MOLARS | 800.51                      | 116.75 | 813.05 | 115.46 | 794.12                            | 113.44 | 812.27 | 128.00 | 813                                    | 126  | 777   | 114  |
| L.R.S.M.                                          |            | 787.74                      | 113.13 | 811.50 | 140.54 |                                   |        |        |        |                                        |      |       |      |
| U.L.S.M.                                          | U.S.MOLARS | 814.13                      | 110.91 | 811.85 | 136.45 | 820.18                            | 112.47 | 854.12 | 131.15 | 852                                    | 129  | 828   | 132  |
| U.R.S.M.                                          |            | 825.00                      | 113.71 | 853.38 | 125.86 |                                   |        |        |        |                                        |      |       |      |



## APPENDIX "H"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: A histological study of tooth germ development both intra- and extra- alveolar with a survey of pathological changes in the developing tooth

Grantee: Dr. J.W. Abbiss  
Dr. A.G. Nutlay

Project No. DR 27 Amount of Grant: \$1,000.00

#### SUMMARY OF WORK

The plan of work in this investigation has been split up into two parts, (1) a study of normal oral and dental embryology and histology, and (2) pathological oral and dental embryology and histology. Up to the present the work has been confined mainly to the first part of this plan, and has consisted in preparing histological slides of foetal and infant jaws obtained in the course of the routine work of the Institute of Pathology. Some twenty-five specimens have now been obtained and preliminary studies on sections of them made, but it is probable that serial sections will also have to be prepared from this material. Difficulties are encountered in preparing satisfactory sections from such material, particularly using paraffin methods of embedding, but now that the sledge microtome purchased under the grant, has been obtained, it is hoped that the double embedding technique, which is not often used in this country, may prove more satisfactory.

A short paper by the grantees, dealing with dental and oral histo-pathology is now ready for publication in the Journal of the Canadian Dental Association.

It is hoped that in a few months' time the second part of the plan, which deals with the histology of retained and displaced tooth germs, may be commenced.

J.W. Abbiss  
A.G. Nutlay







## APPENDIX "I"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: Study of closure of space following premature loss of deciduous teeth

Grantee: Dr. Norma Ford Walker

Project No.: DR 30

Amount of Grant: \$1,200.00

#### SUMMARY OF WORK

Within the scope of this study, not only the closure of space following premature loss of deciduous teeth is investigated, but also the approximate eruption time of the permanent dentition, the width of the growing arches, and the movements of the first permanent molar. This study depends upon a group of measurements taken from models by means of a travelling microscope whose accuracy reaches one hundredth of a millimeter. A maximum of ten measurements are taken from a model which has had no extraction. Eight measurements are commonly obtained from a model of an extraction case. The change of space is shown by three measurements taken from the cuspid to the mesiobuccal cusp, the mesiolingual cusp and the central fossa of the first permanent molar. The movement of the first permanent molar can be observed by the measurements of this space and width of the arch.

Thirty-five children in Toronto public schools have been observed every three months for a period of one year and eight months. A total of 520 models and x-ray pictures have been collected and studied. The results of this study were submitted as a thesis for the master degree of of science in dentistry (on a Red Cross Scholarship).

During the past summer, under the National Research Council, the seventh recall for these children has been completed, including oral examination, impressions, x-rays, preparation of models and measurements. Considerable time has been spent in checking the errors of measurements which are caused by the technic used in marking the models with red dots and in preparation of the models.



A check of the reproductive technic was carried out as follows:

Six sets of impressions were taken from one jaw at one time, and poured with a mixture of plaster and stone of the same consistency. The models were prepared in the same way and the dots (marking points for measurement) were carefully put on as usual. Three sets of measurements were taken by (1) the microscope and (2) the caliper which measures to 1/10 mm. Each distance was measured three times, and one set was repeated six times to check for any variation caused by the microscope. The difference between the six readings on the same models taken with the microscope lay in second decimal point (maximum deviation .04 mm., minimum .01 mm., average .02 mm. and standard deviation .01 mm.), but the difference between the same measurement taken from different models lay in the first decimal point (maximum deviation .35 mm., minimum .01 mm., average .12 mm., and standard deviation .18 mm.) The difference between the measurements taken by caliper was the maximum .20 mm., minimum 10 mm., average 12 mm., and standard deviation .22 mm. The difference between the measurements taken by the two methods was the maximum .24 mm., the minimum .01, the average .09 mm., and the standard deviation .22 mm. From these results, it is known that the reproductive error lies between .01 mm. to .35 mm.

In order to save time and motion, it is reasonable therefore to use an instrument with a scale which can measure to 1/10 mm., instead of the travelling microscope. An instrument has therefore been designed to meet all the requirements for this study. It consists of two sharp pointers and a scale attached by a vernier, which are settled horizontally on two posts. The joints of the posts and the scale are removable and the length of the pointer is adjustable, so that the pointer can actually touch the dots on different levels and the vertical distance between dots can be obtained. Future work will be done with this new instrument.

Since most of the children being studied are 8 or 9 years old, the final results cannot be obtained in a short period of time. Longer observations are required and the work planned for this year is as follows:

1. To observe each child every three months for:
  - a. Closure of space.
  - b. Eruption of permanent dentition
  - c. Growth of the arches
  - d. Movement of the first permanent molar.



"I-3"

11. To measure directly with the new instrument, the extent of drift of the cuspid and the first permanent molar after the deciduous molars are extracted.
111. To repeat with the new instrument the measurements previously made and to compare the results.

The last comparison is important since the writer has argued in her thesis that the rotary movements of an individual tooth could be determined from certain measurements. Whether the variations in these measurements were real or due to errors in the levelling of the models can now be checked by the new instrument.

SUMMARY OF WORK

Inasmuch as I have just arrived at the University of Toronto and undertaken my duties in the Faculty of Dentistry at this place, the research project DR 26 has

September 27, 1949. (Submitted by Dr. Liu)

Norma Ford Walker  
Signature

September 25, 1949

Robert E. Moyers





## APPENDIX "J"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: Measurement of physiologic forces in the mouth

Grantee: Dr. Robert E. Moyers

Project No.: DR 26                      Amount of Grant: \$1,300.00

#### SUMMARY OF WORK

Inasmuch as I have just arrived at the University of Toronto and undertaken my duties in the Faculty of Dentistry at this place, the research project DR 26 has not yet been started. Therefore, no report is possible.

There is every reason to believe that it will be started at once and I am looking forward to submitting a complete report at a later date.

September 29, 1949

Robert E. Moyers





APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: An investigation of the effects of certain chemicals  
on materials in appliances used in prosthetic dentistry.

Grantee: Dr. O. J. Walker and Dr. H. R. MacLean

Project

No.: DR 9

Amount of Grant: \$300.00

SUMMARY OF WORK

It has been demonstrated that Hygeol corrodes cobalt chromium alloys at various temperatures and at several concentrations. Polident and Sterakleen do not corrode the alloys under the same conditions.

The research on this project has been written up in an article that is to be submitted for publication. Two copies of the paper will be sent along in a day or so. The grantees request the Publication Committee of the Associate Committee on Dental Research to clear the paper for publication. It is suggested that the Journal of the Canadian Dental Association be the first choice. The copies sent to the Committee should be forwarded to the editor of the Journal chosen.

Date: 30 September, 1949.

O. J. Walker

Signature





APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1949

Subject: Determination of small amounts of fluorine by means of the photoelectric colorimeter.

Grantee: Dr. O. J. Walker

Project

No.: DR 28

Amount of Grant: \$1000.00

SUMMARY OF WORK

The Sanchis method and Scott's modification of the Sanchis method have been found applicable to a photoelectric colorimeter. The Lumetron photoelectric colorimeter 402 E with a 15 cm. tube was used. Both of the above methods employ the bleaching effect of fluorides on a zirconyl-sodium alizarin sulfonate lake. Results are good to 0.1 p.p.m.

Scott's method was found to be applicable to the determination of fluorides in the range: 0 p.p.m. to 1.4 p.p.m.

Sulfates fail to interfere with the determination up to 120 p.p.m., but greater than this, every 300 p.p.m. of sulfate causes an apparent increase in fluorine content of 0.1 p.p.m.

Phosphates do not interfere up to 2 p.p.m.

Scott's method is more time-saving than that of Sanchis.

The effect of higher concentration of phosphates will be studied next. Following this, work will be carried out on material containing organic matter.

September 30, 1949.

O. J. Walker  
Signature.





## APPENDIX M

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1949

Subject: The effects of hard and soft diets on the supporting structures of the teeth

Grantee: Dr. C.H.M. Williams

Research worker: Dr. D.G. Watt

Project No.: DR 24 Amount: \$3,080.00

In a previous report to the committee, the experimental procedure was fully described. Since that time, the dried mandibles of the rats have been completely examined and the following findings are presented:-

#### (a) Wear:-

No wear was observed on the occlusal surfaces of the molar teeth of the animals fed the soft diet, while a moderate degree of wear was present in the animals fed the hard diet. The wear was most prominent on the first molar tooth, the cusps being almost obliterated and the occlusal surface flat. The second molar tooth exhibited less wear, the cusp height being reduced by about half. Still less wear was shown by the third molar tooth. The tips of the cusps were just worn through, exposing the dentine.

There was no change in the pitch of the occlusal plane as a result of wear. In both groups a typical Monson's pitch was observed.

#### (b) Radiographic findings:-

All the mandibles were radiographed, using a constant technique as to exposure and development. The radiographs were examined directly and also indirectly, by studying photographic prints that had been enlarged three times. No difference in the density of the bone between the two groups could be established by this method, with the exception that the articulating surfaces of the condyles of the soft diet group were less radiopaque than the hard diet group.



(c) Weight, Volume and Density Analysis:-

The mandibles were dried and immediately weighed. It was found that except in three pairs, the mandible of the soft diet animal was lighter than that of the hard diet animal. The mean weight of the mandibles of the soft diet group was .0284 gms. or 7.77% less than those of the hard diet group. A t test of significance <sup>\*</sup> was applied to the data, resulting in a t value of 7.35. Therefore, the difference in the weights of the mandibles is a significant one.

The volumes of the mandibles were estimated by immersing them in a small jar containing mercury and in many respects the volume data is very similar to that pertaining to the weights of the mandibles. Except in six pairs (including the three exceptions in the weight analysis), the mandible of the animal fed the soft diet was less in volume than the mandible of the animal fed the hard diet. The mean volume of the soft diet mandibles was .016 cc. or 7.02% less than the hard diet mandibles. The t value was 6.52 and therefore the difference in volume is significant.

It will be observed that the average difference in the weights and volumes of the mandibles is of the same order and also, where the difference in weight in the individual pairs is great, the difference in volume is correspondingly large. This observation led to a density analysis and a figure of the average density of the bone was obtained by dividing weight by volume. This figure is remarkably constant for all the mandibles and the difference between the two groups almost negligible. The difference amounted to 0.01 grams/cc., the soft diet mandible being 0.62% less dense than the hard diet mandible. A t test was again applied to this data and this difference was found to be not significant.

(d) Planimetric Analysis:-

This area analysis was carried out on a three times enlargement of the radiograph of each mandible. In order to ascertain more accurately where any change in area had taken place, the mandible was divided into three areas in the following manner. A perpendicular was drawn from the inferior

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\* The critical value of t at the .001 probability level for 29 degrees of freedom is 3.66



border of the bone to the distal aspect of the distal root of the third molar tooth. This line was continued along the distal surface of the root to the superior border of the ramus. Similarly, a perpendicular was drawn from the lower border of the mandible to the mesial aspect of the mesial root of the first molar tooth and this line was continued along the mesial root surface to the superior bone edge. Thus, the mandible was divided into three areas - a posterior portion, area A, largely under the influence of the muscles of mastication, a central area, area B, including the molar teeth, a portion of the incisor and the supporting bone, and an anterior portion, area C, consisting of the anterior section of the incisor tooth and its supporting bone.

The total area of the buccal aspect of the mandibles of the soft diet group was found to be, on the average, 4.2% less than the hard diet group and this difference could be accounted for, almost entirely, by a change in the area posterior to the molar teeth. The difference in the total area and in the portion of the mandible posterior to the molar teeth were found to be statistically significant, but there was no difference in the central and anterior portions of the bone.

(e) Width of the Mandible:-

The total bucco-lingual width of the mandible was measured in the region of the distal root of the first molar tooth, mid-way vertically along the root. Except in one pair, the width of the mandible of the soft diet animal was less than the mandible of the hard diet animal. The mean difference was .006 in. or 6.17%. This difference is a real one, the t value being 10.2.

(f) Analysis of the Angle formed by the Occlusal Plane and the Inferior Border of the Mandible:-

During the study of the photographic prints taken from the radiographs, it was observed that a change had taken place in the angle formed by the occlusal plane and the inferior border of the mandible. Consequently, this angle was measured and it was found that in every case except two pairs, the angle was greater in the soft diet mandible than in the hard diet mandible. The mean difference amounted to 2.7°.



"M-4"

Table 1.

Summary of the weight, volume and density analysis

|                 | Hard diet animals | Soft diet animals | $\bar{d}$ | %age d. | t    |
|-----------------|-------------------|-------------------|-----------|---------|------|
| Average Weight  | .3655 gm.         | .3371 gm.         | .0284 gm  | 7.77%   | 7.35 |
| Average Volume  | .228 cc.          | .212 cc.          | .016 cc.  | 7.02%   | 6.52 |
| Average Density | 1.61gm/cc         | 1.60gm/cc         | 0.01gm/cc | 0.62%   | 1.25 |

Table 2.

Summary of the planimetric study

|             | Hard diet animals | Soft diet animals | $\bar{d}$  | %age d. | t.   |
|-------------|-------------------|-------------------|------------|---------|------|
| AREA A.     | .492 sq.in        | .462 sq.in        | 0.30 sq.in | 6.1%    | 4.62 |
| AREA B.     | .268              | .264              | .004       | 1.5%    | 1.60 |
| AREA C.     | .192              | .188              | .004       | 2.1%    | 1.50 |
| TOTAL AREA. | .951              | .911              | .040       | 4.2%    | 4.20 |

Discussion of Data:-

The significance of wear of the teeth has been emphasized by many writers, but there are a number of controversial opinions. Wear is a continuous process operating throughout the life of the animal. It brings about an even distribution of function and thus a good mechanical situation is created which is an important factor in the maintenance of periodontal health.



The analysis of the weight and volume of the mandibles is very interesting. The mandibles of the animals that were given the soft, pappy diets, were lighter and smaller than the mandibles of the animals fed the hard diet, but yet their average density remained the same. This differs from the opinion of those who maintain that increased function causes an increase in the density of bone without any alteration in form. It may be thought that the differences are only slight, but three factors must be kept in mind. Firstly, the experiment was carried out for only a four-month period; secondly, the difference in the degree of functional activity due to the alteration in the physical consistency of the food was not great; and thirdly, the incisor teeth of the soft diet group were not mutilated. The real difference between the two groups was apparently to be found in the load on the molar teeth and the associated muscular activity.

The planimetric study of the mandibles tends to show that the form of the mandibles had been affected by a change in masticatory function. This was apparently due to the activity of the muscles of mastication, because the area difference was confined to the region posterior to the molar teeth where the majority of these muscles are attached. No difference was to be expected in the remainder of the mandible, for this area is largely occupied by the incisor and the molar teeth.

The difference in the width of the supporting bone about the molar teeth is likely significant. Frail development of the supporting structures about the roots of the teeth is considered by some to be an important predisposing cause of periodontal disease. The evidence presented tends to substantiate the claim that the degree of masticatory function is an important factor in this regard.

The change in the angulation of the acclusal plane is presented as a matter of interest. No significance to it can be ascribed.

A microscopic study of the supporting structures about the molar teeth is in progress. Sections have been prepared with the purpose of examining, in detail, the changes in the gingival tissues, the periodontal membrane and the supporting bone. A preliminary study tends to show that there is a marked difference in the degree and type of inflammatory reaction in the gingival tissues. The gingiva of the soft diet group exhibited a marked degree of chronic inflammation, while the hard diet group showed a slight acute



inflammatory reaction, due mainly to the impaction of food fibres. The keratin layer and the epithelium of the gingiva of the animals fed the hard diet were thicker than in the soft diet animals.

This initial experiment is in the nature of a pilot study. It is felt that the findings to date are important, and significant to all those interested in dental health as affected by food intake. The importance of diet in relation to dental health is well recognised, but emphasis is usually placed on the nutritional or chemical constituents of food without due regard to its physical character. This preliminary study tends to show that the physical character of the food is an important factor in the development of the mandible of the rat.

At present two studies are being planned in order to carry the work a stage further. A similar experiment will be conducted over a period of one year, and a smaller group of adult animals will be fed hard and soft food for four months in order to observe the changes that take place in the mandible after the growth period has finished.

C.H.M. Williams



## APPENDIX "N"

### SUMMARY OF INTERIM REPORT

to

#### ASSOCIATE COMMITTEE ON DENTAL RESEARCH

1. Subject: The experimental artificial production of dental caries in vitro.
2. Authors: Dr. H. K. Box, D.D.S., Ph.D. and Dr. R.A.Hugill, D.D.S.
3. Project No.: DR 18
4. Grantee: Dr. H. K. Box
5. Summary of Work

The experimental work investigating the effects of acids and enzymes on human teeth has been continued. Results were duplicated to those of the previous experiments. However, some new facts regarding the problem have been interpreted and so much broader conception of the caries process can be conceived. Reference to this will be made later in the report.

#### (a) Enzyme Experiments

The three enzymes used were pepsin, papain, and trypsin, as well as pepsin, and papain in conjunction. Results showed surface (also slight penetration) pigmentation and minute loss of interprismatic substance with the exception of trypsin action. Papain and pepsin when used together produced more profound results.

#### (b) Acid Experiments

Next, experiments with lactic acid were carried out to determine further effects of acids if possible. Results were identical as those described previously, that is, cross-striation of the enamel rods in early decalcification to complete decalcification with loss of structure in the final stages.

#### (c) Acid-Enzyme Experiments

To follow up these individual experiments with acids and different enzymes, both "factors" were used simultaneously to set up the artificial "Caries mechanism" that has been described in earlier reports. In explanation of the set up, "it" consists of a combined solution of acid (lactic acid principally used) along with proteolytic enzymes (pepsin and papain). It will be recalled that many of the



significant characteristics of the natural caries lesion were duplicated artificially through this means. It is significant to note that when papain was used in conjunction with lactic acid or as a follow-up solution to one combining lactic acid and pepsin enzyme that the pigmentation and organic disintegration or digestion was somewhat more pronounced. This might be expected since papain is capable of breaking down proteins further than pepsin.

#### (d) Further Experiments

At present, experimental work is continuing with the use of lactic acid and pepsin-papain preparations as an approximate "caries-producing mechanism" (sterile solutions). They are being used against possible caries preventive agents such as fluorine, ammoniated dentifrices, oleic acid, and one or two others in order to attempt to test their preventive qualifications against caries. Results will not be interpreted until the next report.

#### (e) Caries Hypothesis

1. The key to caries process involves an enzymatic hydrolysis of enamel and dentin matrix. There are at least four type enzymes participating simultaneously.

Sulphatases - which release the protein radical from the sulphated complex chondroitin sulphate molecule of enamel and dentin.

Proteases - to further the breakdown of the protein molecule of enamel and dentin.

Tyrosinase - enzyme which presumably assists in the pigment formation of caries.

Hyaluronidases - which may assist in the breakdown of connective tissue ground substance (dentin).

2. Although enzymatic action may play a key role in the process, organic, and to some extent inorganic acids liberated through bacterial metabolism and other means, play a vital and intricate part in the same caries process. They are responsible for the dissolution of inorganic carbonates, sulphates, phosphates, etc. of enamel and dentin matrix. They also probably tend to act as catalysts for enzymatic activity, and at the same time limiting the field of enzymatic hydrolysis to those group of enzymes which are capable of activity in an acid pH. medium. It is therefore unlikely that alkaline enzymes such as pancreatic trypsin take part in the enzyme hydrolysis of tooth tissues.



## APPENDIX "N"

### INTERIM REPORT TO

### ASSOCIATE COMMITTEE ON DENTAL RESEARCH

1. Subject: The experimental artificial production of dental caries in vitro.
2. Authors: Dr. H. K. Box, D.D.S., Ph.D. and Dr. R.A. Hugill, D.D.S.
3. Project No.: DR 18
4. Grantee: Dr. H. K. Box
5. Summary of Work

The experimental work investigating the effects of acids and enzymes on human teeth has been continued. Repetition of some of last terms experiments, as described in the last report, have shown results with a marked similarity to the previous results rather conclusive evidence of the authenticity of the findings as reported from the first experiments attempted.

However, some new facts regarding the problem have been interpreted and a much broader conception of the caries process can be conceived. Reference to this will be made later in the report.

First, in consideration of the effects of proteolytic enzymes on human teeth, chiefly 3 enzymes were used, namely pepsin, papain and trypsin. Observations were made on the activity of all three enzymes separately on human enamel as well as the effects of pepsin, activity followed by papain activity on the same teeth. Both pepsin and papain enzymes are known to have an acid pH. around 4.5 while trypsin is activated only in a basic pH. medium.

Pigmentation with minute loss of the inter-prismatic ground substance was in evidence near enamel surface due to both pepsin and papain activity and slightly more pronounced when both enzymes were used simultaneously in conjunction. But, significantly, trypsin and other alkaline enzymes had no apparent effect on tooth enamel. This is an important point as will be established later.

Next, experiments with lactic acid were carried out to determine further effects of acids if possible. Results were identical as those described previously, that is, cross-striation of the enamel rods in early decalcification to complete decalcification with loss of structure in the final stages.



## "N-2"

To follow up these individual experiments with acids and different enzymes, both "factors" were used simultaneously to set up the artificial "caries mechanism" that has been described in earlier reports. In explanation of the set up, "it" consists of a combined solution of acid, (lactic acid principally used) along with proteolytic enzymes (pepsin and papain). It will be recalled that many of the significant characteristics of the natural caries lesion were duplicated artificially through this means. It is significant to note that when papain was used in conjunction with lactic acid or as a follow up solution to one combining lactic acid and pepsin enzyme that the pigmentation and organic disintegration or digestion was somewhat more pronounced. This might be expected since papain is capable of breaking down proteins further than pepsin.

From these experiments, we can feel very sure about several facts concerning the caries process.

1. Acids will not dissolve the organic material of dentin, and although microscopically undetermined, probably are incapable also of dissolving the organic content of enamel.
2. Acids alone do not produce pigmented substance in dental caries.
3. The carious process must comprise of an intermittent or simultaneous enzyme-acid reaction, in which acid and enzyme destroy or digest the inorganic and organic content of enamel and dentin respectively.

What significant bearing have these results on the interpretation of the real nature of the carious process? There are two basic statements regarding the dental caries process that are generally agreed on.

1. The destructive agents are products of bacterial metabolism, ie from organisms most commonly found associated with dental caries.
2. Both the organic and inorganic substance of enamel and dentin are broken down wholly or partially digested or dissolved.

Since the results of these experiments show that the effects of intermittent or simultaneous acid and enzyme attack are the essential phases of the caries process, it is obvious that they must chemically represent the constituent destructive metabolic products from the bacteria associated with the caries picture.

In the past, too much emphasis has been placed upon the importance of decalcification (by acids) as the principal phase of the caries process, while the enzymatic phase was rather ignored or disregarded to some degree. But



this fact does not coincide with the findings that most mouths, including those moderately immune to totally immune register acid pH's in the saliva. Why then if free acid is so prevalent in the mouth that teeth do not decalcify more readily than they do ?

Therefore, it is not unreasonable to presume that more emphasis, than that hitherto, should be placed upon the importance of the enzyme phase of the caries process. Suppose we look upon caries as essentially a process of enzymatic chain destruction with acid decalcification a vital but secondary factor. Since the knowledge of specific enzyme activity has so advanced in recent years, it is not incredible to believe that tooth enamel and dentin could be broken down to a considerable extent by a series of bacterial enzymes.

There are a few important facts found in recent literature that might be advantageously recorded here.

1. Pincus states that quantitative estimations of nitrogen and sulphur together with evidence from the developmental aspect and from histochemical tests suggest that enamel protein is a mucoprotein.
2. A substrate resembling chondroitin sulphuric acid by qualitative tests has been prepared from dentin (Pincus).
3. From this substrate (chondroitin sulphuric acid) enzymes of gram negative bacilli in pure culture have been capable of quantitative release of sulphate.
4. Chondrosulphatase (a bacterial sulphatase) is capable of hydrolizing or releasing sulphate from chondroitin sulphuric acid.
5. Bacteria are also capable of releasing an enzyme tyrosinase which is capable of oxidizing tyrosin (a constituent of enamel and dentin protein) to a brownish black pigment called melanin.
6. Hyaluronidase enzyme often referred to as the "spreading factor" of infection, acts on and hydrolyses the ground substance of connective tissue. Certain bacteria are capable of liberating this enzyme when metabolizing on a suitable substrate.

Thus a different explanation or hypothesis of caries development in enamel and dentin may be as follows:

Enamel protein resembles a mucoprotein in properties. Gram-negative bacilli commonly found in caries can release sulphatase (possible chondrosulphatase). Enamel



is made up of hexagonal prisms each surrounded by a sheath of enamel protein. Enzymatic hydrolysis of these sheaths in caries, with release of sulphuric acid, would result in attack on each prism from the periphery inwards: the relatively insoluble calcium phosphate which forms a considerable portion of the inorganic part of the enamel would be slowly changed to the relatively soluble calcium sulphate. It is of interest to notice that Frisbie shows enamel caries proceeding in just such a manner, each prism decreasing in size from the outer edge inwards. The pigmentation associated with dental caries in enamel and dentin has been shown to be an insoluble melanin type pigment accompanied by other soluble pigments. Bacterial tyrosinase quite likely plays an important role in the production of melanin pigment. Hyaluronidases also probably contribute to the caries process, assisting in the break-down of the ground substance of connective tissues, (dentin). Bacterial proteases and acid tryptases such as papain must assist in the organic protein digestion of enamel and dentin. Although enzymatic action may play a key role in the process, organic and to some extent possible inorganic acids, liberated through bacterial metabolism and other means, play a vital and intricate part in the same caries process. They are responsible for the dissolution of inorganic carbonates, sulphates, phosphates etc. of enamel and dentin matrix. They also probably tend to act as catalysts for enzymatic activity and at the same time limiting the field of enzymatic hydrolysis to those group of enzymes which are capable of activity in an acid pH. medium. It is therefore unlikely that alkaline enzymes such as pancreatic trypsin take part in the enzyme hydrolysis of tooth tissues.

Such a hypothesis cannot be disregarded or turned aside without intensive investigation. It is the author's hope that such a project would be supported in the very near future.

At present, experimental work is continuing with the use of lactic acid and pepsin-papain preparations as an approximate "Caries-producing mechanism" (sterile solutions). They are being used against possible caries preventive agents such as fluorine, ammoniated dentifrices, oleic acid, and one or two others in order to attempt to test their preventive qualifications against caries. Results will not be interpreted until the next report.



APPENDIX " O "

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1949

Subject: Chemical mechanisms in dental caries

Grantee: J. J. Rae (C. T. Clegg)

Project

No.: DR 1

Amount of Grant: ...\$1,000....

Period Covered: Feb. 8, 1949 to Sept. 30, 1949

SUMMARY OF WORK

The work covered during this period falls into three parts.

- (a) Salivary Amylase - J. T. Dentay
- (b) Ammonia Production in Saliva - C.T. Clegg
- (c) Organic Fluorides.

(a) The work on salivary amylase was completed and permission to publish these results will be sought from the committee. (Report to follow).

(b) Saliva on standing produces ammonia but we were unable to show any marked difference between low l.b.a. count and high l.b.a. count salivas in this regard. A study of ammonia production in saliva has yielded the following results:-

- (1) A period of 24 hours is sufficient to measure the amount of ammonia produced.
- (2) The optimum pH for this mechanism is 8.5
- (3) Centrifuging and filtering reduce the amount of ammonia production considerably which would indicate that it is partly due to bacteria.
- (4) Shaking saliva with permutit removes the ammonia producing mechanism and we have not been able to elute the mechanism by means of water, alcohol, trichlor acetic acid, NaCl and  $\text{Na}(\text{PO}_4)_3$  solutions.
- (5) The ammonia producing mechanism is inhibited by boiling the addition of antiseptics and strangely enough by the addition of glucose.
- (6) The presence of a urease in saliva has been shown and this enzyme is also removed by permutit and inhibited by glucose.  
(Report on this work enclosed)

(c) A start has been made on the synthesis of non-toxic organic fluorides with the hope that these may furnish a more effective agent for the topical application of fluorine.

James J. Rae

Signature







Section B. AMMONIA PRODUCTION IN SALIVA

Kesel has reported that with low lactobacillus acidophilus (l.b.a.) saliva, after 8-days incubation, the filtrate contains a factor that (i) inhibits acid production; (ii) inhibits l.b.a. growth of fresh saliva. He suggests that ammonia is the active ingredient since low l.b.a. count saliva produced ammonia whereas high l.b.a. count saliva produced little or no ammonia on incubation.

If this is so a difference in ammonia production on incubation of low and high l.b.a. count saliva should be observed and there should be a mechanism in low l.b.a. count saliva that produces ammonia which is absent or negligible in amount in high l.b.a. count saliva. With these thoughts in view the following experiments were done in an attempt to study the mechanism and amount of ammonia production in high and low l.b.a. count salivas.

EXPERIMENTAL

(1) The ammonia production of high and zero count salivas was studied and the results are shown in Table I.

TABLE I

| The relation between ammonia production and l.b.a. count of saliva |                                |                                    |                               |                                |                                    |
|--------------------------------------------------------------------|--------------------------------|------------------------------------|-------------------------------|--------------------------------|------------------------------------|
| Low l.b.a.                                                         |                                |                                    | High l.b.a.                   |                                |                                    |
| 0 hrs<br>mg % NH <sub>3</sub>                                      | 24 hrs<br>mg % NH <sub>3</sub> | Difference<br>mg % NH <sub>3</sub> | 0 hrs<br>mg % NH <sub>3</sub> | 24 hrs<br>mg % NH <sub>3</sub> | Difference<br>mg % NH <sub>3</sub> |
| 1.29                                                               | 38.6                           | 37.3                               | 1.29                          | 19.1                           | 18.8                               |
| 6.18                                                               | 51.5                           | 45.3                               | 9.95                          | 42.8                           | 32.8                               |
| 20.6                                                               | 54.0                           | 33.4                               | 10.32                         | 39.8                           | 29.5                               |
|                                                                    |                                |                                    | 8.38                          | 28.4                           | 20.0                               |
|                                                                    |                                |                                    | 7.74                          | 31.0                           | 23.3                               |
|                                                                    |                                |                                    | 2.5                           | 30.8                           | 28.3                               |



There appears to be no marked differences in ammonia production between high and low l.b.a. count salivas. There seems to be a tendency for high l.b.a. count salivas to produce less ammonia on incubation than low l.b.a. (299, 301, 306, 321).

(2) The relation between time and ammonia production is shown in the following graph (Graph 1). From this graph 24 hours seems to be a suitable time to measure differences in rate of ammonia production (315).

(3) The effect of pH on ammonia production was studied by determining the amounts of ammonia produced at various pH's in well-buffered solutions. The results indicate that the optimum pH for this mechanism is about 8.5 (314, 327, 328, 345).

(4) The next procedure to be investigated was the effect of centrifuging the saliva for 15 minutes at 2200 r.p.m. and filtering the saliva through a No. 1 filter paper with suction on the ability of the saliva to produce ammonia.

If either of these treatments inhibits ammonia production it would seem likely that it was due to bacterial action.

(4a)

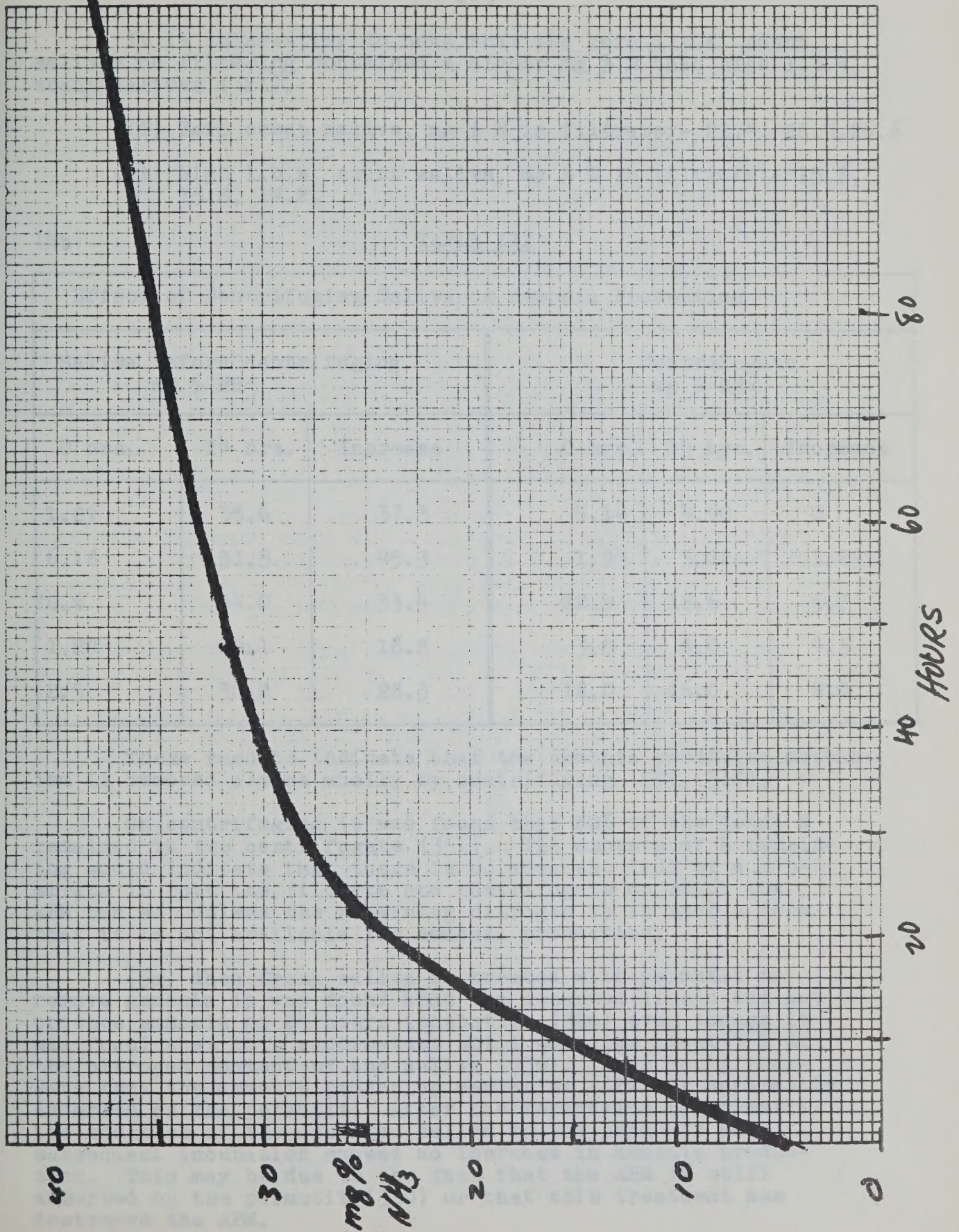
TABLE II

| Effect of Filtering saliva on Ammonia Production |                                                 |         |          |                                  |          |
|--------------------------------------------------|-------------------------------------------------|---------|----------|----------------------------------|----------|
|                                                  | Saliva Before Filtering<br>mg % NH <sub>3</sub> |         |          | Filtrate<br>mg % NH <sub>3</sub> |          |
|                                                  | 0 hrs.                                          | 24 hrs. | Increase | 24 hrs.                          | Increase |
|                                                  | 6.46                                            | 41.7    | 35.2     | 3.36                             | 0        |
|                                                  | 2.58                                            | 58.0    | 55.4     | 0.91                             | 0        |
|                                                  | 3.87                                            | 44.2    | 40.3     | 3.23                             | 0        |
|                                                  | 5.80                                            | 32.0    | 26.7     | 11.4                             | 6.62     |

Filtering saliva through a Seitz filter with the disc covered with a thin layer of celite (Hyflo Supercel) removed the ammonia producing mechanism since on subsequent incubation of the filtrate no further increase in ammonia was observed (317, 321, 346). Analyses showed however, that about one third of the total N of the saliva still remains in the filtrate.



Figure I









"0-3"

It is interesting to note that the zero l.b.a. count salivas on filtering contained a higher mg % N than high l.b.a. count salivas (321).

(i) Zero count saliva, mg % N in filtrate:- 21.4, 28.3, 26.6

(ii) High l.b.a. count saliva, mg % N in filtrate:- 15.8, 15.8, 14.2.

(4b)

TABLE III

| Effect of Centrifuging Saliva on Ammonia Production |         |          |                                      |         |          |
|-----------------------------------------------------|---------|----------|--------------------------------------|---------|----------|
| Saliva before centrifuging<br>mg % NH <sub>3</sub>  |         |          | Centrifugate<br>mg % NH <sub>3</sub> |         |          |
| 0 hrs.                                              | 24 hrs. | Increase | 0 hrs.                               | 24 hrs. | Increase |
| 1.29                                                | 38.6    | 37.3     | 5.14                                 | 4.90    | 0        |
| 6.18                                                | 51.5    | 45.3     | 1.94                                 | 3.22    | 1.29     |
| 20.6                                                | 54.0    | 33.4     | 12.9                                 | 18.6    | 5.7      |
| 1.29                                                | 19.1    | 18.8     | 3.5                                  | 8.0     | 4.5      |
| 2.5                                                 | 30.8    | 28.3     | 12.0                                 | 16.0    | 4.0      |

These results indicate that the ammonia producing mechanism is removed almost wholly by centrifuging (299, 318).

On centrifuging it was found that 80% of the total N remained in the centrifugate (351). The amounts of N remaining would indicate that there is no apparent lack of a substrate in both the filtrate and centrifugate on which the APM can act unless the remaining nitrogen is in such a form that it is not available for ammonia production.

(5a) When fresh saliva is treated with permutit to remove ammonia it was found that the decanted liquid did not produce ammonia on 24 hours incubation (326) even though it was found that this supernatant still contains about 4/5 of the nitrogen content of the saliva (336). This would indicate that the ammonia producing mechanism (APM) is apparently adsorbed on the permutit. After the separation by permutit the addition of the decanted saliva to the permutit and subsequent incubation showed no increase in ammonia production. This may be due to the fact that the APM is still adsorbed on the permutit (330) or that this treatment has destroyed the APM.



(a) When the saliva was shaken with permutit and centrifuged, the elutriate obtained by washing the permutit with NaOH after neutralization with HCl and mixing with the original centrifugate, produced no ammonia on incubation (332).

(b) In another attempt to elute the APM from the permutit 10% alcohol was used, but it was found that this elutriate when added to the permutit supernatant also did not produce ammonia on incubation (340). Saliva containing a comparable per cent of alcohol showed that the alcohol did not inhibit the ammonia production. Another experiment (342) using as elutriates higher percentages of alcohol gave the same result. Alcohol does not seem to be an effective reagent for removing APM from permutit.

(c) In view of the fact that it has been stated that washing of permutit with 2% acetic acid makes the permutit fit for re-use it was thought advisable to attempt to elute the APM with this reagent. When this was done it was found that no ammonia was produced by adding the acetic acid elutriate after neutralization by NaOH, to 5 ml. of the permutit treated saliva (344).

Further attempts to remove the APM from permutit with 8% NaCl, 8% Na<sub>3</sub>PO<sub>4</sub> and 10% t.c.a. proved unsuccessful (350). In each case the solutions used were adjusted to pH 8.0 before adding the original saliva and incubating for 24 hrs.

(5b) When saliva was shaken with Kaolin and then centrifuged the centrifugate after 24 hours incubation showed a considerable increase in NH<sub>3</sub>. The saliva and Kaolin when incubated after shaking for 5 minutes showed an even greater increase in NH<sub>3</sub> production. This behaviour is quite different from that observed with permutit. Kaolin is not as effective as permutit in removing the APM from saliva (337, 341).

(6a) The addition of Thymol to the saliva inhibited the production of ammonia on incubation (323, 324). The addition of merthiolate partially inhibited the production of ammonia. These results would indicate that the APM may be partly bacterial in action.

(6b) Heating saliva for 5 minutes at 99° and subsequent incubation completely inhibited ammonia production on subsequent incubation for 18 hours at 37°C. (325).

(6c) The addition of 0.2% glucose to a group of low l.b.a. count and high l.b.a. count salivas inhibited almost completely the production of ammonia on 24 hours incubation at 37° (302). This rather remarkable effect is to be investigated further.



(7) Urea and Urease. Since urease is an ammonia producing enzyme it was thought advisable to see if there were any urease in saliva.

The addition of urease (Jack Bean Meal Extract) to saliva showed that there was no urea present (333, 334).

The addition of urea to saliva (1 ml. 3% urea to 5 ml. of saliva) and subsequent incubation at 37° showed a much greater production of ammonia than saliva without the addition of urea. This would indicate the presence of a urease in saliva (338, 343).

On 24 hours incubation the results obtained with two different salivas, A and B, were:-

(i) With urea added A:- 129 mg %  $\text{NH}_3$ ; B:-219 mg %  $\text{NH}_3$

(ii) With no urea added A:- 25.8 mg %  $\text{NH}_3$ ; B:-23.8 mg %  $\text{NH}_3$

When the time of incubation was reduced to 1 1/2 hours the following results were obtained:

(i) With urea added A:- 28.4 mg %  $\text{NH}_3$ ; B:- 19.3 mg %  $\text{NH}_3$

(ii) With no urea added A:- 15.0 mg %  $\text{NH}_3$ ; B:- 6.4 mg %  $\text{NH}_3$

Urea itself under the conditions of the experiment produced no ammonia.

Since the presence of urease in saliva has been established it would be interesting to know how this enzyme behaves when treated in a similar manner to the APM in saliva. For example is urease removed on shaking with permutit? Does the addition of glucose also inhibit urease? Experiments have shown that in these particulars at least, urease and APM behave in an analogous manner, i.e. the supernatant obtained by shaking a urease extract with permutit showed no urease activity (353) and the addition of 6% glucose inhibited ammonia production by urease from urea (354).

### SUMMARY

(1) When saliva is incubated ammonia is produced and contrary to the findings of Kesel there seemed to be little, if any, difference in ammonia production by either low or high l.b.a. count salivas.

(2) Ammonia production reaches a maximum in about 40 hours so that 24 hours seems to be a suitable time in which to study the ammonia-producing-mechanism (APM).



(3) The optimum pH for this mechanism is about 8.5.

(4) Vigorous centrifuging and filtering inhibits the APM although 50-75% of the nitrogen is still present in the saliva after this treatment.

(5) The addition of thymol and heating at 95° for 5 minutes also destroys the APM.

(6) Strangely enough the addition of 0.2% glucose also inhibits ammonia production.

(7) When saliva was shaken with permutit the resulting centrifugate did not produce any ammonia on incubation. The addition of the washed permutit to the treated saliva did not restore the APM. Attempts to elute the APM with sodium hydroxide, alcohol and acetic acid have so far proved unsuccessful. Kaolin when used as an absorbent did not remove the APM.

(8) One APM occurring in the body is urease acting on urea. The addition of urease to saliva showed the absence of urea but the addition of urea showed the presence of a fairly active urease in saliva which may in part account for the APM.



## APPENDIX "P"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: Biological absorption of fluorine

Grantee: Dr. M.D. Smith

Project No.: DR 29      Amount of Grant: \$1500.00

#### SUMMARY OF WORK

As well as the preliminary work on DR 29, the completed analyses of DR 14 are reported. Since baking powders are an important source of fluorine, they were included. Both a single distillation method (Godfrey & Shrewsbury, Jour. A.O.A.C., 28: 335, 1945) and a double distillation method (Methods of the A.O.A.C. 1945) were attempted with phosphate baking powder with rather inconclusive results. A single and a double distillation procedure were also carried out with the combination baking powders and the results seemed to justify the use of a double distillation with this type.

| <u>Substance</u>                                | <u>Fluorine content</u>           |
|-------------------------------------------------|-----------------------------------|
| Coffee infusion                                 | 0.88 micrograms fluorine /100 ml. |
| Toronto water                                   | 0.12, 0.11 p.p.m.                 |
| Coca-cola                                       | 0.06, 0.07 p.p.m.                 |
| Breakfast (Juice, Cream of wheat, toast, marm.) | 1.04, 0.77 p.p.m. (dry weight)    |
| Dinner - salad meal                             | -----                             |
| - beef, macedoine etc.                          | 0.11, 0.26 p.p.m.      "      "   |
| - halibut, spinach etc.                         | 1.04, 0.92 p.p.m.      "      "   |
| Pabena (baby food)                              | 11.3, 12.1 p.p.m.                 |
| Bonemeal (capsules)                             | 246, 285, p.p.m.                  |
| Soluble fluorine in Pabulum                     | app 10% at 40° C. for 15 min.     |
| Royal B.P. - (tartrate)                         | 1.36, 1.34 p.p.m.                 |
| Magic B.P. - (phosphate)                        |                                   |
| - single distillation                           | 6.0, 6.1 p.p.m.                   |
| - double distillation                           | 5.9, 14.0, 4.7, 0.84 p.p.m.       |
| Calumet B.P. (combination)                      |                                   |
| - single distillation                           | 19.1, 12.4, 15.3, 16.4 p.p.m.     |
| - double distillation                           | 25.0, 32.0 p.p.m.                 |

Preliminary experiments were performed to measure accurately the amount of fluorine in the atmosphere by bubbling the air through 0.5 N NaOH, but further work is necessary on this aspect.



"P-2"  
(Summary)

In a continuation of work begun in the summer of 1948, vegetables were grown where the fluorine content of the atmosphere was believed high (glass factory) and where it was lower.

| <u>Vegetable</u> | <u>Fluorine content (p.p.m. dry weight)</u> |             |                      |             |
|------------------|---------------------------------------------|-------------|----------------------|-------------|
|                  | <u>Ave. Rd. &amp; Bloor</u>                 |             | <u>Glass factory</u> |             |
|                  | <u>1948</u>                                 | <u>1949</u> | <u>1948</u>          | <u>1949</u> |
| Spinach          | 112, 117                                    | 60.2, 56.4  | 154, 169             |             |
| Lettuce          |                                             | 73.7, 52.2  |                      |             |
| Beet greens      |                                             | 34.2, 36.3  |                      | 36.0, 36.0  |
| Beets            |                                             | 0           | 0                    |             |

Poplar leaves were also analyzed at different periods this season to see if the fluorine content was increasing as suggested by Churchill, et al. (J. Ind. Eng. Chem. anal. 20:69, 1948)

| <u>Leaves</u> | <u>Fluorine content (p.p.m. dry weight)</u> |                  |
|---------------|---------------------------------------------|------------------|
|               | <u>May, 1949</u>                            | <u>Aug. 1949</u> |
|               | 17.7, 18.7                                  | 19.6, 13.0       |

In preparation for the work on absorption of fluorine by humans, the method for fluorine determination was modified to be applicable to urine and some analyses done. The urine analyzed contained 0.5 p.p.m. fluorine. Balance studies on infants are now being planned.

September 29, 1949.

M. Doreen Smith  
Signature

Preliminary experiments were performed to measure accurately the amount of fluorine in the atmosphere by bubbling the air through 0.5 N NaOH, but further work is necessary on this aspect.



## APPENDIX "p"

### Report to the Associate Committee on Dental Research

October, 1949

**Subject:** Biological absorption of fluorine

**Grantee:** Dr. M.D. Smith

**Project No.:** DR 29      **Amount of Grant:** \$1500.00

Project DR 29 is an outgrowth of DR 14 which was concerned with the fluorine content of various foods. This report completes the analyses of the foods studied and gives preliminary work on project DR 29.

These foods included a coffee infusion which contained 0.88 micrograms fluorine /100 ml. as compared with tea infusions which were quite high in fluorine--91.6-127.0 micrograms /100 ml. Toronto water was found to have 0.12, 0.11 p.p.m. fluorine and coca-cola 0.06, 0.07 p.p.m. fluorine.

A breakfast, (orange juice, cream of wheat, toast, marmalade), a salad meal and a hot dinner with beef and macedoine vegetables etc., were analyzed and compared with a fish and spinach etc., dinner already reported.

| <u>Meal</u>          | <u>Fluorine Content</u> |                      |
|----------------------|-------------------------|----------------------|
|                      | <u>p.p.m. dry wt.</u>   | <u>Total - (mgm)</u> |
| Breakfast - as above | 1.04, 0.77              | app. 0.10            |
| Dinner - salad, etc. |                         |                      |
| - beef, macedoine,   |                         |                      |
| etc....              | 0.11, 0.26              | app. 0.03 mgm        |
| - halibut, spinach,  |                         |                      |
| etc....              | 1.04, 0.92              | app. 0.15 mgm        |

Pabena, a baby food similar to Pablum was also found to have a high fluorine content--11.3, 12.1 p.p.m.

Bone meal capsules, "Bone Aid" obtained from the research department at Wayne University were found to contain 246, 285 p.p.m. fluorine.

Baking powders are an important source of fluorine and in Canada in a cooked food where baking powder is used, a maximum of 1.4 p.p.m. fluorine has been considered. In Britain, baking powders may not contain more than 100-130 p.p.m. fluorine. The determination on phosphate baking powders involved a double distillation with perchloric acid (Methods



of Analysis of the Association of Official Agricultural Chemists, 1945) since the phosphates may interfere with the titration to give high results. However, Godfrey and Shrewsbury (Journal of the Association of Official Agricultural Chemists, 28:335, 1945) recommend a single distillation for the determination of fluorine in minerals and this was also attempted. The determination of fluorine on the combination baking powder containing aluminum which represses the evolution of fluorine, necessitated a preliminary distillation with sulphuric acid followed by a distillation with perchloric acid. Single distillations were also attempted on this type.

| <u>Baking Powder</u>  | <u>Fluorine, p.p.m.</u> |
|-----------------------|-------------------------|
| Royal - (tartrate)    | 1.36, 1.34              |
| Magic - (phosphate)   |                         |
| - single distillation | 6.0, 6.1                |
| - double distillation | 5.9, 14.0, 4.7, 0.84    |
| Calumet (combination) |                         |
| - single distillation | 19.1, 12.4, 15.3, 16.4  |
| - double distillation | 25.0, 32.0              |

The results with the phosphate baking powder are inconclusive and seem to indicate an unreliability of the method with this substance. The results with the combination baking powder seem to justify the use of double distillation.

It had been suggested by Dr. Rae at the 9th meeting of the Associate Committee in Dental Research that the determination of water-soluble fluorides as distinct from calcium fluorides should be made. It was found that the fluorine in Pabulum is approximately 10% water soluble at 40° C. for 15 min. It seems that at high levels of intake calcium fluoride is far less toxic to the rat than sodium fluoride, (Smith and Leverton, J. Ind. Eng. Chem., 26:791, 1934), but at low levels of intake such as are usual in food, the form in which fluorine exists in the food probably makes little difference to its absorption and toxicity (Smith and Leverton, 1934; Lawrenz, et al. Journal of Nutrition, 18:115, 127, 1939). Machle and Largent, (J. Ind. Hyg. & Tox., 25:112, 1943) and McClure et al. (J. Ind. Hyg. & Tox., 27:159, 1945) have found that at low levels of intake approximately 60% of fluorine in calcium fluoride in food and 40% of that in bonemeal is absorbed from food as compared to approximately 90% from sodium fluoride in food.

Since it was felt that the method of measuring atmospheric fluorine was not satisfactory (see previous report) further work was done on this aspect of the problem. The absorption agent used was 0.5 N. NaOH (Enamelist, p.5, Jan. 1949) and a method of bubbling a known amount of air through



this agent to give an accurate measurement of the fluorine content of the atmosphere was devised with the assistance of Dr. Katz, Defence Research Board, Ottawa. However, in the preliminary experiments the results showed no appreciable absorption of the fluorine by the agent from the atmosphere. Further work on increasing the efficiency of absorption agents is planned.

In a continuation of the work begun in the summer of 1948, vegetables were grown in the same soil in an area where atmospheric fluorine was believed to be high (glass factory) and one where it was lower. Chiefly because of an unfavourable growing season, samples were not obtained in all cases as desired.

| <u>Vegetable</u> | <u>Fluorine content (p.p.m. dry weight)</u> |             |                      |             |
|------------------|---------------------------------------------|-------------|----------------------|-------------|
|                  | <u>Ave. Rd. &amp; Bloor</u>                 |             | <u>Glass factory</u> |             |
|                  | <u>1948</u>                                 | <u>1949</u> | <u>1948</u>          | <u>1949</u> |
| Spinach          | 112, 117                                    | 60.2, 56.4  | 154, 169             |             |
| Lettuce          |                                             | 73.7, 52.2  |                      |             |
| Beet greens      |                                             | 34.2, 36.3  |                      | 36.0, 36.0  |
| Beets            |                                             | 0 0         |                      |             |

Although spinach and lettuce seeds, and soil were the same in both districts, those at the glass factory did not grow. One cannot conclude what the unfavourable condition or conditions were which caused this failure. The beet greens in both districts showed a similar fluorine composition and both showed negligible fluorine in the roots.

Poplar leaves were also analyzed at different periods this season to see if the fluorine content was increasing as suggested by Churchill, et al. (J. Ind. Eng. Chem. Anal., 20:69, 1949).

|        | <u>Fluorine content (p.p.m. dry weight)</u> |                  |
|--------|---------------------------------------------|------------------|
|        | <u>May, 1949</u>                            | <u>Aug. 1949</u> |
| Leaves | 17.7, 18.7                                  | 19.6, 13.0       |

Another analysis will be done in October.

In preparation for the work on the absorption of fluorine by humans, especially children, a number of fluorine analyses on urine were completed. Preliminary experiments resulted in high acid distillates which had to be discarded, but on the addition of excess of 10 gm. of silver sulphate to precipitate the chlorides, a determination could be completed, although this amount of silver sulphate rather complicates the procedure. The urine analyzed contained 0.5 p.p.m. fluorine.

Preparations are being made to attempt fluorine balance studies on infants before and after Pablum and other solid food are added to the diet.







APPENDIX "Q"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario.

Grantee: M. Doreen Smith

Project

No.: DR 13

Amount of Grant: \$1,475.00

SUMMARY OF WORK

The fluorine content of teeth extracted from individuals living in Kitchener, a low fluoride area and Stratford, a comparatively high fluoride area, has been studied. Fluorine determinations were made on whole teeth using the modified Willard and Winter technique. In general, it would appear that Stratford teeth have a higher fluorine content than Kitchener teeth. The trend for the fluorine content of teeth to increase with age was also noted. Differences between results for sound and carious teeth could not be correlated.

Analyses were also carried out on teeth from young dogs who had been treated with topical applications of 2% sodium fluoride. An average increase in the fluorine content of the teeth from .032 to .099 per cent was noted after 8 treatments at weekly intervals.

The Manly and Hodge technique for separating teeth into enamel and dentin was applied to a number of teeth extracted from Brantford children. The separated fractions were analyzed for fluorine content by the chemical method. The ratio of fluorine in dentin to fluorine in enamel was estimated to be 2.9 for permanent teeth and lower for deciduous teeth.

Work on the microbiological technique of fluorine analysis has also been carried on, an attempt having been made to apply the method to samples of urine. The results are more promising for urine than were obtained previously with teeth. However, further work should be done to increase the accuracy of the method.

October 3, 1949

M. Doreen Smith  
Signature







# APPENDIX "Q"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario

Grantee: M. Doreen Smith

Project No.: DR 13 Amount of Grant: \$1,475.00

### Fluorine Content of Teeth from High and Low-Fluoride Areas

Because of the possible significance of the fluorine content of teeth to their resistance to dental caries, a study was made of teeth extracted from individuals living in relatively high and low fluoride areas. The cities of Stratford (1.25 to 1.33 p.p.m. fluorine in municipal water supply) and Kitchener (0.12 to 0.19 p.p.m. fluorine in water supply) were chosen as representative areas so that the work might be correlated with a study previously made of the fluorine content of water and milk from these two communities. Teeth from two other areas have also been analyzed and the results included for purposes of comparison. These are Ripley, Ont. reported by Box and Hodgins(1) as a high fluoride area and the Falkland Islands, about whose water supply no information was available. All determinations were made using the modified Willard and Winter chemical method.

### Results

TABLE I

### Fluorine Content of Whole Teeth

| No. | Age | Sex | District         | Tooth Analysis                                         | Degree of Caries | Per Cent Fluorine |
|-----|-----|-----|------------------|--------------------------------------------------------|------------------|-------------------|
| 1   | -   | -   | Falkland Islands | perm. upper central incisors; pooled sample of 2 teeth | none             | 0.052(9)          |
| 2   | -   | -   | Falkland Islands | perm. upper central incisors; pooled sample of 2 teeth | moderate         | 0.050(2)          |



TABLE I (cont'd)

| No.             | Age                                  | Sex | District        | Tooth Analysis                                    | Degree of Caries                                                                                 | Per Cent Fluorine |
|-----------------|--------------------------------------|-----|-----------------|---------------------------------------------------|--------------------------------------------------------------------------------------------------|-------------------|
| 3               | Child -<br>age &<br>sex un-<br>known |     | Ripley,<br>Ont. | deciduous molar,<br>crown only                    | none                                                                                             | 0.012(0)          |
| 4               | 15                                   | M   | Ripley,<br>Ont. | permanent molar                                   | moderate<br>caries but<br>exhibits<br>mottling as<br>did other<br>teeth in<br>patient's<br>mouth | 0.071(4)          |
| 5               | 11                                   | F   | Stratford       | deciduous<br>second molar<br>L.R. (crown<br>only) | slight                                                                                           | 0.013(2)          |
| 6               | 7½                                   | F   | Stratford       | permanent first<br>molar U.R.                     | showed sl.<br>decalcifi-<br>cation                                                               | 0.031(4)          |
| 7               | 18                                   | M   | Stratford       | permanent first<br>molar                          | severe but<br>showed<br>mottling                                                                 | 0.054(6)          |
| 8               | 22                                   | F   | Stratford       | permanent 2nd<br>molar                            | slight                                                                                           | 0.029(4)          |
| 9 <sup>3</sup>  | 42                                   | F   | Stratford       | pooled sample<br>of 2 permanent<br>bicuspid       | none                                                                                             | 0.039(8)          |
| 10 <sup>3</sup> | 42                                   | F   | Stratford       | permanent 2nd<br>bicuspid                         | none                                                                                             | 0.049(2)          |
| 11              | 10                                   | -   | Kitchener       | deciduous 2nd<br>molar U.R.                       | severe                                                                                           | 0.0075            |



TABLE I (concluded)

| No. | Age | Sex | District  | Tooth Analysis                              | Degree of                    | Per Cent |
|-----|-----|-----|-----------|---------------------------------------------|------------------------------|----------|
| 12  | 14  | M   | Kitchener | permanent first bicuspid                    | none, but showed hyperplasia | 0.013(9) |
| 13  | 15  | F   | Kitchener | permanent 2nd molar                         | moderate                     | 0.0083   |
| 14  | 19  | -   | Kitchener | permanent 2nd molar L.L.                    | moderate                     | 0.0082   |
| 15  | 21  | F   | Kitchener | permanent third molar                       | none; tooth not erupted      | 0.0075   |
| 16  | 25  | M   | Kitchener | permanent first molar L.R.                  | moderate                     | 0.024(7) |
| 17  | 33  | F   | Kitchener | permanent 2nd molar L.R.                    | severe                       | 0.020(5) |
| 18  | 39  | M   | Kitchener | permanent 2nd molar U.R.                    | slight                       | 0.033(1) |
| 19  | 42  | F   | Kitchener | pooled sample of two lower central incisors | none                         | 0.023(2) |

<sup>3</sup>Numbers 9 and 10 not from the same individual

Any comparison of sound and carious teeth made between different individuals for fluorine differences will likely introduce errors due to variation in the individual's fluorine exposure. Values listed in Table I show no significant difference in fluorine content between sound and carious teeth.



There appears to be a correlation between the fluoride content of the water and the percentage fluorine found in the teeth. Not enough results have been obtained for statistical analysis but in general Stratford teeth have a higher fluorine content than Kitchener teeth. These results are in line with the findings of the previous survey by Miss M. Ham with respect to milk. (see DR 14 reports)

Tooth No. 7 (Stratford) had the highest fluorine value of the teeth analyzed from either Stratford or Kitchener, but also showed slight mottling of the enamel.

Tooth No. 4 (Ripley) had the highest fluorine content in the table. Although the tooth was carious, it exhibited mottling as did all of the teeth in the patient's mouth.

The increase in fluorine content of the teeth with age and exposure to dietary fluorides is also shown. The lowest values in Table I are for deciduous teeth and a third molar which had not erupted. Allowing the variations in the individual's fluorine exposure, there is a gradual increase in the fluorine content of permanent teeth with age.

The acquisition of fluorine through topical applications to the teeth of young dogs has recently been investigated in the Faculty of Dentistry of this university. The primary purpose was to see what effect fluorine treatments had on surface microhardness of the teeth, but fluorine analyses of the teeth were also made in this laboratory. Results of fluorine analyses of the teeth from two of the dogs showed an increase of about 200 per cent in the treated teeth over the controls. See Table II.

TABLE II

Fluorine Content of Whole Dogs' Teeth

|       | Control |   | Teeth treated with 2% NaF<br>(8 applications) |   |
|-------|---------|---|-----------------------------------------------|---|
|       | % F     |   | % F                                           |   |
| Dog 1 | .032    | % | .087                                          | % |
|       | .037    | % | .101                                          | % |
|       | .037    | % |                                               |   |
| Dog 2 | .034    | % | .103                                          | % |
|       | .029    | % | .075                                          | % |
|       | .029    | % | .127                                          | % |
|       | .028    | % | .098                                          | % |
| Ave.  | .032    | % | .099                                          | % |



Analysis of Enamel & Dentin Fractions of Teeth extracted from Brantford children

A number of the teeth which were sent to this department by Dr. H.K. Brown, chief of the Dental Health Division, Ottawa, have been separated into enamel and dentin fractions by the Manly and Hodge method. Both fractions have been analyzed for their fluorine content using the chemical method. The values obtained to this date are given in Table III below.

TABLE III

Fluorine in Enamel and Dentin of Brantford Teeth

| Name            | Age | Tooth Analysis                       | Degree of Caries | Enamel % Fluorine | Dentin % Fluorine | Ratio of Dentin to Enamel |
|-----------------|-----|--------------------------------------|------------------|-------------------|-------------------|---------------------------|
| Melvin Freedman | 6   | deciduous lower molar                | severe           | .0067             | .0093             | 1.4                       |
| Selma Gassee    | 7   | pooled sample of 2 deciduous cuspids | none             | .0018             | .0046             | 2.6                       |
| Thelma Minshall | 9   | deciduous molar(crown only)          | moderate         | .0085             | .012(0)           | 1.4                       |
| Gerald Owens    | 10  | deciduous lower first molar          | none             | .015(4)           | .014(3)           | 0.9                       |
| Pearl Merridew  | 12  | permanent upper right first molar    | severe           | .0049             | .011(6)           | 2.4                       |
| June Watson     | 12  | permanent upper first bicuspid       | none             | .0078             | .010(5)           | 1.4                       |
| John Marsh      | 13  | permanent lower first molar          | severe           | .0032             | .016(7)           | 5.2                       |
| John Marsh      | 13  | permanent upper first molar          | severe           | .0062             | .012(4)           | 2.0                       |



TABLE III (concluded)

| Name             | Age | Tooth Analysis                 | Degree of Caries | Enamel % Fluorine | Dentin % Fluorine | Ratio of Dentin to Enamel |
|------------------|-----|--------------------------------|------------------|-------------------|-------------------|---------------------------|
| Annette Davidson | 15  | permanent upper first molar    | severe           | .0029             | .011(4)           | 3.9                       |
| Jos Hayward      | 15  | permanent upper first molar    | severe           | .0036             | .013(6)           | 3.8                       |
| Donald Whyte     | 17  | permanent lower first bicuspid | severe           | .012(3)           | .022(1)           | 1.8                       |

While there are only 4 teeth in the deciduous group, the ratio of fluorine in dentin to fluorine in enamel has a mean of 1.6, whereas the mean ratio for the permanent group is 2.9. (If the high value 5.2 is not taken into account, the mean is 2.55 for permanent teeth). McClure(2) refers to the relatively consistent relation of fluorine in dentin to fluorine in enamel. His analyses on over 500 teeth indicate that dentin seems to contain about 2.0 to 2.5 times as much fluorine as enamel.

From the results above, (Table III), one might believe that there is a tendency for the fluorine content of the dentin to increase in relation to the fluorine of the enamel in permanent teeth.

#### Application of Microbiological Technique of Fluorine Analysis

Since it was hoped to do fluorine balance studies in this department, an attempt was made to apply the microbiological technique of fluorine analysis outlined by L.L. Winter to samples of urine.

The 24 hour collection from one subject was found to be 550 ml. The fluorine content of duplicate 100 ml. samples was determined by the chemical method. To each 100 ml. sample of urine was added 1 ml. of 25% magnesium acetate fixative. The sample was made alkaline, evaporated to dryness, ashed at a temp. of 520° C. and the usual distillation and



titration carried out. To precipitate all of the chlorides contained in 100 ml. of urine it was estimated that 10 g. of silver sulphate would be required. Since this chemical is relatively expensive and tends to cause bumping in the distillation flask, it was felt that it would be advantageous if the microbiological method could be used. Duplicate samples of urine analyzed by the chemical method gave .57 and .59 p.p.m. of fluorine (ave. .58 p.p.m.). The daily output of fluorine would be 319 $\checkmark$  or .319 mg.

The microbiological technique was tried using a second 24 hour sample from the same subject (800 ml. volume). McClure and Kinser (3) found that where domestic waters were free of fluorine, the fluorine present in urine averaged 0.3 to 0.5 p.p.m. Toronto water contains small amounts of fluorine (values of 0.11 and 0.12 p.p.m. have been obtained by M. P. Ham).

A 200 ml. sample of urine was made alkaline to phenolphthalein and evaporated down to a little less than 50 ml. in volume. The pH was adjusted to 5.1 and the volume made up to 50 ml. Originally the urine was a clear straw-coloured liquid but concentration to quarter volume at pH 5.1 resulted in a darker liquid containing a ppt. in fine suspension. Half of this was set aside to be assayed microbiologically. Additional fluorine amounting to 6 p.p.m. was added to the second half. Both solutions were now assayed using the microbiological technique. Using evaporated urine alone, a value of 0.45 p.p.m. was obtained. This would be equivalent to  $(.45 \times 8.80)$  396 $\checkmark$  or .396 ml. fluorine for the 24 hr. period. The urine + 6 p.p.m. added fluorine gave a graph reading of 7.52 where 7.80 p.p.m. was expected. That is the 6 p.p.m. added fluorine was not completely recovered, only 4.9 p.p.m. showed up in the assay.

Evaporation of urine samples had been carried out in platinum dishes but this would not be practical if a large number of analyses were to be made concurrently. A comparison was therefore made of the differences obtained evaporating standard aqueous solutions of sodium fluoride (made alkaline) in platinum and porcelain dishes. Platinum was found to give better results than porcelain, but even these results were not too satisfactory. The assay was carried out at two levels, 4 and 8 p.p.m. Two different experiments with platinum dishes gave values higher than the theoretical (7.6, 10.8) and (7.6, 11.7) p.p.m. which seemed to indicate the presence of some other substance causing inhibition.

A possibility that some contamination of the solution took place when adjusting the pH with the Coleman pH meter was examined. Two assays were carried out, one in which



the pH of the assay sample was adjusted to pH 5.1 with the Coleman pH meter and the second in which this adjustment was made using bromthymol blue indicator. The same amount of this indicator was also added to the first sample before it was made up to volume. The sample adjusted with the Coleman pH meter was 6.3 p.p.m. and with the indicator, 5.8 p.p.m.

The previous experiments raised some doubt as to whether there were sufficient points in the standard reference curve which is usually obtained by taking the best straight line through points 0, 5, 10, 15 and 20 p.p.m. However, from 0 to 5 irregularities have occurred from time to time. Therefore an assay was carried out in the higher range, i.e., using 8 and 16 p.p.m. fluorine in order to see if more reliable results were obtained. Assaying at two levels provides a check on the method. One value was found to be somewhat high (8.6 instead of 8.0) while the other was low (14.9 instead of 16.0). These graph values seem to suggest that inhibition of acid production with graded increments of fluorine may not give exactly a straight line relationship. The tendency for the line to curve very slightly had been noticed in a number of the previous reference graphs.

It was felt that the method showed promise for analyzing urine samples but was in need of further refinement before the desired accuracy could be obtained.

- (1) Bbx, H.K., & Hodgins, H.J., Water & Sewage, May, 1944.
- (2) McClure, F.J., J. Dent. Res. 27:287, 1948.
- (3) McClure, F.J., and Kinser, C.A., U.S. Pub. Health Rept. 59:49, 1944.

M. Doreen Smith  
Signature



APPENDIX "R"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949.

Subject: Improvement of sub-gingival curettage and gum resection techniques

Grantee: Dr. Jules Thébaud, D.D.S.

Project

No.: DR 20

Amount of Grant: \$750.00

SUMMARY OF WORK

Our experiments and investigations have been discontinued from March 10th to June 20th, 1949, as renewal was officially granted during the first part of June (correspondence from April to June 1949).

Curettage of the cemental wall - To complete our series of decalcified specimens already examined in January, 27 more slides were prepared and studied. 13 other teeth involved with periodontal pathosis and on which experiment was conducted, were examined lately. 11 ground sections were also mounted and studied.

Curettage of the soft tissue at the periodontal wall and at the base of the pocket - Arrangement has been made with Notre-Dame Hospital in Montreal, in order to obtain specimens for biopsy from autopsy material, within not less than 2 hours after death. Complete report will be made in our final study, next February.

Improved technique in gum resection (gingivectomy) - Further experiment is being carried out with our calibrated pocket marker, for which some improvement brought up much more accuracy.

Our periodontal micrometer was mailed to the Officer in Charge of Patents of the National Research Council in Ottawa, for examination and further consideration.

Gingivectomy with our new armamentarium will be performed for a few more patients, in order to record the various steps of our technique by means of lantern slides or silent movies, in compliance with our application for this project.

Date: 3 October, 1949.

Jules Thébaud  
Signature.







## APPENDIX "S"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1949

Subject: A study of alveolar oxygen tension and blood oxygen content during nitrous oxide-oxygen anaesthesia.

Grantee: Prof. G. H. W. Lucas

Project

No.: DR 2

Amount of Grant: \$1600.00

#### SUMMARY OF WORK

A method has been devised by Dr. R.J.S. Tickle employing the Fry Gas Analyser to measure accurately the oxygen and carbon dioxide content in 1 cc. of inspired and expired nitrous oxide-oxygen mixtures during surgical anaesthesia in dental operations. The gas for analysis was collected in small glass syringes lubricated with liquid paraffin. Owing to a certain amount of rebreathing into the gas machine, the inspired gases frequently contained small quantities of carbon dioxide. The carbon dioxide content of the expired gases was measured to ensure proper sampling. The somewhat surprising result, namely that the expired gas contained (in a number of cases when low percentages of oxygen were given) more oxygen than the inspired, suggested that the packing in the mouth was inadequate or that oxygen was leaving the blood which was becoming more reduced. Measurements using the oximeter to determine the blood oxygen content would offer the best means of deciding this point. It is suggested that the research should be continued, if facilities such as apparatus and suitable patients can be secured. At present no further funds are required for this research as Dr. Tickle has discontinued it. It is hoped that an application to continue the work will be filed in February 1950.







### Trends in Caries Research.

A few years ago Rosebury (371) stated that it is difficult and perhaps impossible to distinguish with any degree of assurance between fact and fancy in the literature of caries, to sort the grain of verified or verifiable knowledge from the chaff of prejudiced observation and idle speculation. A flimsy, but well guarded, structure of prejudice has grown up about the whole subject, much of it will have to be broken down before the truth becomes appreciable. The foundation of the problem must rest securely in a knowledge of the form, structure and chemical composition of the teeth and their relationships to one another and to their environment. Dental literature is overstocked with papers that attempt to explain caries by a purely speculative approach and which offer conceptions difficult, or impossible, to verify or contradict by experiment.

There have been many clinical experiments on caries, but very few have been carried out with a proper appreciation of the problems involved. Thelander (1555) warns against the use of loosely constructed human control experiments. Where multiple factors are involved, most of which are beyond control, the result from controlling one factor is masked by the influence of the uncontrolled factors. Natural variations may offset experimental constants. The human animal does not lend himself to laboratory controlled experiment, which has been accepted as almost the only prototype for a study of clinical laboratory problems.

The size and composition of test and control groups, particularly when working with humans, has too often led to erroneous conclusions. There has been too often a faith in numbers. The thought that if a sufficiently large number of individuals is selected that errors in tabulation of results will be averaged out. The identification of the caries lesion in the mouths of both groups is a painstaking procedure which can only be carried out to the required standards on relatively small groups. This error has been referred to by Schlack (731) when he showed that inaccurate initial examination gives a quite different picture in the results of experiments in that it shows an apparent higher rate of caries increase than where the initial examination for caries is accurately carried out.

A large number of human experiments have been reported on the basis of the change in the number of carious lesions at beginning and end of experiment. There are so many variables involved in the examination for such lesions that the accuracy of the claims of many workers is subject to question. The carious lesion is slow in development and at some indefinite point passes from the stage where it is only visible with the aid of a microscope to the point where it may be detected by accepted clinical methods. There is not now, nor is it likely that there will be in the future, methods for measuring the rate of growth of the microscopic stage of the lesion, since the teeth must be sectioned. It is probable that rate of growth in







this and later stages of the lesion varies within wide limits. This means that there will be considerable variation of opinion among different workers, and even at different times by the same examiner, as to what constitutes a developing lesion. This personal factor is further complicated by the difficulties of the clinical examination. In this connection a number of workers have reported interesting figures. Arnet (1739) found that 40.6% of lesions are missed when the x-ray is not used. Deatherage (1629) found a variation of from 0.19 to 2.13 teeth per child in examinations made under as nearly identical conditions as possible by five different operators. Sognnaes (747) showed progressive accuracy in examination by drying the teeth, by cleaning and drying and by the addition of the x-ray. Burket (711) compared histological examination with the results of a combined clinical (mirror and probe) and x-ray examination on a cadaver and found that 24% of the lesions were only detectable microscopically. Barr (1381) found that 43.96% of approximal cavities were not detected by clinical examination without the x-ray. Chilton (1347) found an average increase of 1.05 cavities per child when x-ray added to mirror and probe examination. There is marked general agreement between all these reports and it would appear from this that the accuracy of many claims for benefit from various forms of treatment is based on faulty clinical examination.

The necessity of control groups is well recognized, but some workers have neglected to provide controls; while others as Burrill (520) in his claims for vitamin K, have compared their results with groups having no connection with the experiment. Two methods of control have been used. In one, a comparable group of humans to those undergoing the test are selected; while the other, which has been widely used in topical fluoride applications, makes use of the untreated quadrants of the mouth as control. Both methods have limitations. The untreated quadrant is only suitable for a limited class of experiment. There has been rather general acceptance of the idea that where control groups of humans are used the two groups should be comparable in age, sex and living conditions. It is essential that test and control groups should only differ from one another in the single respect of the experimental factor which is under investigation. It is almost impossible to secure such rigid control with humans in more than some quite limited types of experiment. Further, it is questionable whether the usual conditions of sex, age and living conditions are enough. The available evidence would seem to indicate that the progress of the carious lesion is variable and may be accelerated or retarded by factors which are not explainable at present. It would seem essential that groups should be large enough to smooth out these individual irregularities in both groups and at the same time they must not be so large that examinations cannot be carried out with great care. Many workers start out with the assumption that test and control groups may be selected by age and sex from institutional children living under similar conditions. There is an implied belief here that the two groups so selected are strictly comparable, but this is taking too much for granted. It is apparent that many workers are







in too much of a hurry to publish results and have not carried on their experiment over a sufficiently long period. It would seem necessary that careful records should be kept of each group for a year before the experiment started. The experiment should be carried on for a sufficiently long period, of perhaps a year, and both groups should be kept under observation for a year after conclusion of the test.

These limiting factors in "in vivo" experiments on caries have been recognized and efforts have been made to supplement them by "in vitro" and animal experiments.

There have been quite a few attempts to produce "in vitro" caries, but very few of these have made a contribution to caries research. A number of workers have secured decalcification, or even destruction of the tooth structures under conditions quite dissimilar to those obtaining in the oral cavity and have called these lesions caries. There are some marked differences in these lesions, both in microscopic appearance of the lesion and in its location on the tooth, from carious lesions in the mouth. The experiments conducted by such workers as Miller (1784), Howe (287), McIntosh (227, 234), Clark (232), Bunting (245) Anderson (313) and others, resulted simply in acid destruction of tooth material and should be recognized as such. Pincus (505) seems to have been the first to obtain destruction of an enamel area in a neutral medium. The more recent work of Dietz (860) and the California group of Frisbie, Hurst, Nuckolls and co-workers (1780, 2273) are a big advance over earlier work in this field. These men use a more accurate nomenclature and speak of the production of caries-like lesions. If anyone has attempted to produce an "artificial mouth", there is no reference to it in the literature, yet this would not seem to be any more difficult than the "artificial heart", "artificial kidney", etc. It is possible to produce a synthetic "saliva" which closely approaches the normal fluid. This fluid could be directed through a chamber, corresponding to the mouth, in which the natural teeth are placed in position and which is equipped with motor driven brushes to imitate action of tongue and cheeks. It would then be possible to observe action of microorganisms singly, or in groups, under conditions simulating those of the mouth.

The third main method of experimentation has been by the use of animals. Mrs. Mellanby, in her long series of experiments on diet and tooth structure, used the dog. She chose this animal because it can be kept healthy in the laboratory environment and can eat and live on food similar to man. However, the dog is highly caries-resistant and for that reason not satisfactory. Rosebury (371) pointed out that no experimental animal can be identical with man and that two criteria must be met: (1) The animal species must be suitable in point of practical similarity to man, and (2) the experimental disease must be essentially the same as in man. He thought that the rat, among common animals, appeared to most nearly fill these requirements. However, it was Rosebury who was one of the first to point out that it was not until the rat had been used for some time as a subject for caries research that the lesion in the rat was not strictly comparable to that of man, in that particle size and hardness of the food is an







important factor in rat caries. The early work on the rat was done mostly on the white rat, later the cotton rat was more favored by some writers. The Syrian hamster has been used by a number of recent workers. It still has not been proved that the lesion in these animals is strictly comparable to that of man. The monkey has been used by some workers and, while there are some strong arguments for the use of this animal, there is the difficulty that his natural diet is dissimilar to that of man.

There have been a number of attempts to describe or define the dental caries lesion, but so far none of these are completely acceptable. Some workers use the terms "etched" and "decalcified" areas and consider that these are not true caries lesions. Most caries research has been directed at the clinically perceptible lesion, but it should not be overlooked that the lesion may have advanced for some distance into the tooth before it can be detected by ordinary clinical methods, including the X-ray. Thus the lesion may be said to have a microscopic, or pre-clinical, and a macroscopic or clinical stage. When a worker refers to a patient as caries-immune, or caries-susceptible, he is stating that the patient either had not, or has, clinically discernable caries. It has already been stated that Burket (711) found a 24% increase in caries when teeth were sectioned and examined under the microscope and while these figures only relate to one case our knowledge of caries leads to the conclusion that all patients, other than true caries-immunes, will have caries present in their mouth in the pre-clinical stage. It is further probable that most, at least, of these lesions will at some indefinite time in the future, be recognizable by clinical examination. There is thus a definite objection to the term caries-immune, as so frequently used in the literature. The term caries-resistant would more correctly describe these people. It has only been in recent years that much study has been directed to the pre-clinical stage of the lesion. Frisbie (519), Gottlieb (1621) and Applebaum (1986), by examination under the microscope, and Wolf (2027), by the collodion replica technique, have studied the pre-clinical stage of caries at a time when the lesion would not be detectable by the usual clinical methods. Study of the earliest phase of the lesion has been too much neglected and should be actively pursued. There has been quite general acceptance of a theory, not supported by acceptable proof, that caries started and continued, at least through the enamel as a purely decalcification process. Frisbie (519) and Gottlieb (1419) support their claims that the initial lesion is proteolytic in nature by some excellent photomicrographs. This question is important and should be studied. It is possible that there is not a fixed pattern and that the attack pattern may vary under different conditions.

The pigmentation which has been noted in caries, particularly of the dentin, and which may vary from a faint yellow to a dark brown or almost black color, has been stated by Gottlieb (1590) to be a characteristic of the lesion and if there is no pigmentation, it is not caries. Gottlieb (548, 802) thought that the invading organism produced the pigment. Deakin (589) found no evidence that the pigmentation was due to a pigment-producing organism. It would seem







more likely that this pigmentation is in the nature of a by-product of the lesion and has no direct causal connection with it. The color change may well be simply an oxidation or degradation change of the organic fraction of the enamel. It is much more marked in the dentin, with its larger percentage of organic material. There is also the marked deepening of the color in slowly advancing, or retarded, caries. The observation by Pincus (1530) that enamel changes color when heated, while apatite does not, appears to confirm this. Dreizen (2208) considers that the pigment is a degradation of the organic portion of the tooth.

The publication by Miller (1784) of his classical work on microorganisms of caries marked the scientific approach to caries, although some very good guesses had been published previously. Miller considered that caries was a chemico-parasitic process, consisting of the two stages of decalcification and dissolution of the softened residue. He thought that the acid was derived from starches and sugar. While the caries lesion may cause destruction in enamel, dentin and cementum we are chiefly concerned in caries research with the attack on enamel, since it is in this tissue that most of the lesions start. When Miller published his book, the enamel was considered as inorganic tissue and it was not until Bodecker (284) published his celloidin technic that the presence of a continuous network of organic tissue in the enamel was demonstrated. This belief by Miller that it was only necessary to account for the destruction of inorganic material in the enamel completely dominated caries investigation for many years. There were few workers until recent years who even questioned that enamel caries was anything other than a decalcification process. Investigation was largely confined to the problems of building more resistant teeth, neutralization of the acids formed on, or near, the teeth and mechanism of acid formation. It is difficult today to understand the single minded concentration of so many workers during much of the first half of this century. When Bodecker and others established the presence of the organic sheath surrounding the enamel rods it should have received more attention. The removal of the rod sheaths has simply not been referred to by the "decalcification" school, perhaps on the assumption that the organic fraction was relatively insignificant and could be overlooked, or that it was removed by mechanical action. It is certainly not permissible just to overlook an unexplained factor, nor to assume that this tissue is removed by some form of mechanical action. The latter may be a partial explanation in large, exposed cavities, but an explanation of the mechanism of caries must also apply to protected caries located on approximating surfaces, well protected from all types of mechanical action. These cause extensive destruction of tooth tissue before they become accessible to mechanical action. It is almost as difficult to follow the reasoning of the "proteolytic" school who consider that the organic fraction is removed by bacterial action and the liberated crystals of calcium phosphate, forming the enamel rods, drop out and are removed. It is easy to agree that the main destruction of enamel







may be the result of acid attack of the chief constituent of this tissue. It is difficult, on physical grounds, to see how acid attack could proceed more than a very slight distance when it acts only on one end of a microscopic rod of inorganic material, surrounded by the acid-resistant enamel matrix. If, on the other hand, we postulate an accompanying action in which the organic fraction is also attacked, exposing the rods more completely, then acid destruction is more readily explained. There is the further difficulty that active invasion of microorganisms into tooth tissue can only be by proliferation. Therefore it seems that it must occur along routes where the organism can obtain a food supply. This theoretical approach to the problem then pre-supposes that attack on the organic portion is in advance of the decalcification process. Is it possible that the observations of the many small lesions on the enamel surface shown by Scott (2209) means that these are limited attacks on surface portions of the organic fraction, which do not progress further unless conditions arise in the mouth which favor growth of acidogenic organisms and the lesion then progresses to "clinical caries"?

There has been over emphasis on the acid forming bacteria in the past thirty years and this is directly related to the prevailing concentration on decalcification. It would seem necessary to direct attention to the mechanics of the actual start of the lesion. Is the initial destruction caused by acid-forming or proteolytic organisms? Is it a fixed pattern of attack which always follows the same definite steps, or is the pattern of attack a variable one? It would seem questionable whether this can be answered either by human or animal experiments.

The decalcification theory of caries has played so prominent a role in caries literature during the past sixty years that it can be used as a thread to sew together the story of caries investigation during this period. One of the first things noted in a survey of the caries literature is its marked cyclical nature. The emphasis for a period will be on saliva, this gives way to a wave of papers on diet with heavy emphasis on vitamin D, then perhaps acid-forming bacteria, then fluorides and so on.

Williams (1122), in 1897, stated that he had made over 400 sections of enamel in every stage of decay and always found a thick felt-like mass of organisms overlying the surface where decay commenced. There was little reference in the literature to this "bacterial plaque" or "mucus plaque" for years. In 1923, Williams (271) advanced the thought that the plaque acted like a sponge to hold carbohydrate materials and at the same time protect the acid-forming bacteria in their destructive work. In the interval between these two papers, hundreds of reports of investigations appeared and most of them were quite unrealistic in their approach to the problem in that these workers assumed that the saliva was in direct contact with the carious lesion. These workers knew that the lesion was very slow growing and that there must be some mechanism by which these initial lesions were isolated







and protected from free contact with the saliva for extended periods. This is not by any means the only example in caries investigation to date, where workers rushed into what looked like a promising field, without any apparent attempt to study the fundamental aspects of the problem. The practice of blindly following a lead and rushing into print with unverified theories and conjectures has been much too general. This has been opposed by too little sound, constructive criticism. The net result has been that contributions to our knowledge of dental caries for the fifty year period between Miller's book and the late thirties are small. There are few names which stand out in that period. The importance of the plaque in the initiation of caries was overlooked for a long period, since the method of preparation of sections resulted in destruction of the plaque. The work of Williams (1122), Dietz (860), Frisbie (519) establishes the importance of the plaque.

Most attempts to create plaques have been by in vitro methods as those of Kirk (1161), Bunting (955), Sullivan (497) and Muntz (837). Ennever (2017) has recently suggested an interesting method for development of in vivo plaques.

The plaque would appear to have a rather complex role in caries. It does not follow that caries will develop under a plaque, but rather, that caries can only develop under such a type of covering. This covering also acts as an osmotic membrane. Jones (1269), Bod-ecker (949), Dobbs (78), Enright (77), Pincus (1130), and Bruckner (1848) have investigated this. A number of workers, including Stephan (799, 1547) and Muntz (1508) have shown that sugar solutions can pass rapidly inward through the plaque. The role of the plaque as a buffer itself, or as a membrane permitting passage of acid or neutralizing solutions has received some attention and more work is necessary on this. The emphasis has been on acid formation, with very little investigation of the plaque in connection with proteolytic action.

Study of the organisms of the plaque in relation to caries presents similar problems to the study of the oral flora as a whole. It is likely that there is much the same association of organisms actually involved in the formation of the lesion and of other oral organisms which are in the plaque, but have no part in formation of the caries lesion. Blayney (687), Dietz (860), Frisbie (519), Hemmens (1323), Hurst (1780), and Harrison (1912) have studied the organisms of the plaque.

The relation of the plaque and its organisms to acid formation has received considerable study in recent years by Stephan (351, 799, 899, 1547), Miller (781), Muntz (1508) and Stralsford (1630, 1631). There has been at the same time investigation by a number of workers on inhibitory action in the plaque. These include Grove (146), Stephan (835, 836) and Muntz (837). There has at last been recognition of the role of molecule size of the buffers and the definite attempt by a number of workers to concentrate on the factors present at the site of the caries lesion while it is in its earliest stage.







The early investigations on diet were carried on in the hope that caries might be decreased by helping nature build teeth which were more resistant to acid action. The long series of investigations and articles on "adequate diets", vitamins, etc, which was at its peak in the twenties is one of the most amazing in the whole story of caries investigation. There can be no question that it did much to center attention on the whole problem of diets and, considered from that angle, a fine, constructive bit of work was done. However, we are concerned with the relation of these diet investigations to dental caries. It is too easy to look back and criticize early work from the standpoint of present knowledge, instead it is necessary to inquire whether a particular study was reasonable and in line with the known facts at that time.

When the first of the many publications of May Mellanby appeared, the accepted explanation of the carious lesion was that it was a decalcification process. Therefore, if more of the inorganic components of tooth tissues could be supplied and utilized it was assumed that the individual would have teeth of better structure and more resistant to caries. The most that could be expected of this would be that teeth would be attacked more slowly by acids, it could not "prevent" caries, as was so frequently stated. There were available at that time reports by careful workers showing that primitive peoples, living on diets which certainly could not be considered as adequate, had teeth that were almost caries-free. These people tended to continue with a very low caries incidence until they came into contact with modern civilized food, when caries usually increased rapidly. There is a better understanding today of the ages of calcification of the human teeth, but even at the start of these diet investigations there should have been a realization that it was quite impossible to attempt to obtain better calcification of the crowns of most of the permanent teeth after six years of age. Black had published his reports on the important sites of caries and had definitely related these sites to areas of the teeth which tended to favor food retention. His findings, in this respect, have stood the test for many years. The diet group disregarded this pattern of the incidence of caries in relation to protected surfaces and substituted for it a new pattern, which was determined by structure. This would mean that the smooth, habitually clean surfaces of the teeth would have an incidence of caries similar to say, the approximating surfaces of teeth, if quality of calcification were the predominating factor in the localization of caries. This is quite contrary to clinical experience. It has been observed that where teeth are rotated out of their usual position in the arch that cavities may then form on surfaces which are habitually caries-free. There was the further observation available at that time that teeth of notoriously poor enamel structure were no more subject to caries than well formed teeth.

It was inevitable that calcification and vitamin D would be linked together in the attempt to improve tooth structure. Many claims were advanced, but no evidence has been produced, that the systemic action of vitamin D will in any degree lessen the incidence of caries. This does not rule out the possibility of a local effect in the mouth as a lubricant, when administered in the form of oil.







Schour and Massler (512) state that many careful investigations disprove the idea that caries can be produced, or prevented, by altering the intake of vitamins or minerals. Disturbances of calcification, or of formation of enamel, do not play a significant role in the etiology of dental caries and at best only influence the rate. A balanced diet is no guarantee against caries, conversely, caries is often absent in an individual on an inadequate diet. This statement made in 1945 still holds in so far as the question of tooth structure is concerned, but there is some present indication that vitamins B and K may have a local effect in the mouth. It would appear, at present, that there is no indication of a need for further work on "building better teeth" by diet additions of vitamin D or inorganic salts by their administration either pre-natally or post-natally, where a good diet is consumed.

Carbohydrates have received considerable attention in many studies. If acids are important, then the source of these acids is also important. While reports are available of destruction of tooth tissues by addition of acid to animal diets, or excessive use of lemon juice by humans, these are relatively unimportant and the main source of acids is the carbohydrates. The relative importance of sugars and starches is debatable. Some sugars are rapidly converted into acid in the mouth and will cause a several point drop in pH in a few minutes. Where these sugars are in solution or in a readily soluble form, they are quickly cleared from the mouth by swallowing. Where the sugar is in a less readily soluble form, as hard candies, it may remain in the mouth for long periods. Starches do not provide acid as quickly, but in refined form they may adhere to teeth and provide a source for acids over long periods. Jay (797) and others have reported dietary experiments where the incidence of caries, and of lactobacilli, were reduced as a result of the elimination of a large part of the refined carbohydrates of the diet. On the other hand, there are some people who can consume large amounts of this type of diet and be caries-free. Volker (1586) thinks there is need for data on rate of clearance of carbohydrates from the mouth. Stephan (1827), on the basis of work by McClure (528), Schweigert (1607), Anderson (1838), and Keyes (1467) thought that the common sugars, with possible exception of lactose, are conducive to high caries rates and where the polysaccharides are the sole constituent of the diet that the tendency is to a moderate, or very slow incidence of caries. Sognnaes (2003) recently advanced a new theory in that he thinks it disputable whether caries producing effect of high sugar diet is primarily caused by an unfavorable effect on the oral environment or whether effect is on character of teeth themselves.

The decreased caries incidence of peoples on primitive diets has been reported frequently. The usual explanation has been that this is due to the absence of refined starches and sugars. This is an obvious difference, but that it is the correct explanation remains to be proved. Mann (1363) and Dreizen (1930) have both recently reported low incidence of caries in children on diets deficient in vitamins A, B and C. There is not as yet any explanation of this but there has







been some tendency to link this with vitamin B shortage, either because deficiencies of the vitamin B complex may interfere with carbohydrate degradation in the mouth, or because gingivitis is frequently associated with vitamin B deficiency and end products of protein break down may affect the living requirements of acid-forming organisms. The question is an interesting one.

The enamel has received considerable attention in relation to incidence of carious lesions. Scott (1772) has shown by his collo-dion replica technic that enamel is not the smooth polished surface which it appears to be on visual examination. He (2209) found no difference between the enamel surfaces of non-carious teeth, non-carious surfaces of carious teeth, or the intact enamel portions of carious surfaces. In all cases the areas of intact tooth surface immediately adjacent to the affected regions appeared like normal non-carious surfaces in that particular age group. His comment that it is hazardous to assume that the action of chemical agents upon tooth surfaces is the same as on powdered enamel or on tooth sections is interesting in view of the deductions made in recent years from solubility of untreated, or fluorine, etc treated, powdered enamel.

There have been frequent statements in the literature that the incidence and rate of growth of carious lesions decreases after adolescence. These statements are based on clinical impressions rather than on acceptable scientific data. The available data on incidence of caries is not satisfactory for scientific statistics. It deals mostly with children up to about 16 years of age and there are comparatively few statistics for older age groups. These examinations have usually been carried out for public health purposes, where there is not the same need for the greatest possible accuracy, as required in scientific statistics. The opinion that the general caries curve increases steadily to somewhere about 16-18 years of age and then flattens out to show a slower rate of increase has been made so frequently that it has come to be accepted as fact. The difficulty is that these figures are expressions of the numbers of filled, carious and extracted teeth and a stage is eventually reached where the potential caries sites are decreased to the point that decrease in new caries must follow. It is not possible to obtain accurate figures on rate of caries incidence until figures are available of caries incidence in relation to the number of caries susceptible areas in the mouth. The impossibility of accurately measuring rate of growth of lesions has been referred to already. If there is an actual slowing down of the rate of incidence and growth of caries, there would seem to be some reason for associating this with changes in the tooth tissue. The possibility of change in the inorganic portion of the enamel is confined to limited post calcification physical changes, such as those in fluoride applications. There have been statements that age changes take place in the enamel, but there is no proof of this that can be accepted. These so-called changes are not greater than differences in hardness and composition which have been shown to occur in different teeth in the same mouth. The statement made by Lundstron (1740) that enamel changes in composition, particularly with regard to the magnesium content, cannot be accepted. These analyses were made on teeth of different patients, and







are of such a nature that they are made on extracted teeth. The same criticism applies to reported age changes, with regard to increase in fluorine content. On the other hand, changes in the keratin-like organic fraction of the enamel, as a drying or tanning process, could occur. No record has been found of any investigation of this nature, other than by permeability experiments. Atkinson (1996) has recently stated that the difference in staining between young and old enamel is in the "pore size" of the prism sheath.

Various procedures have been described which were designed to increase surface resistance of the enamel. Gore (456) suggested coating with a special type of nitro-cellulose. Howe and others suggested the use of ammoniacal silver nitrate a number of years ago. While this was generally used on dentin surfaces, the coating of enamel surfaces was also advocated by some. Klein (686) did not find it of value. Gottlieb (602, 1283, 1621), Younger (2111, 2116) and Crawford (2205) suggested various solutions for the purpose of "blocking the organic pathways of the enamel". Zander (1869) and the Council of Dental Therapeutics of the American Dental Association (2098) have both offered criticisms of this which were in part directed at the inadequacy of the supporting clinical data. Atkinson (1995) states that the organic constituent imparts to enamel the property of being able to act as an osmotic membrane and that the applications of silver nitrate, chromium trioxide, etc to surface of enamel does not affect its permeability. It would appear then that more acceptable proof than any yet offered is necessary to establish the value of this form of treatment.

The relationship between enamel solubility and pH level of acid production by mouth organisms has been sought by a large group of workers. It is questionable whether this has made much contribution to the caries study, since there are a number of factors which may enter into the process. Rickert (247) showed that tooth sections are slowly acted on in distilled water. Friesell (241) obtained areas of decalcification on tooth surface in 2-3 days by a stream of water, saturated with carbon dioxide. Stephan (799, 1819) and others place the probable attack level at about pH 5. Brudevold (1867) refers to the effect of protein deposits on solubility of intact surfaces. He made the unexpected finding that solubility of the enamel surface bore no relation to solubility of the surface beneath it. He thinks that this lack of correlation suggests that solubility of the surface depends not upon original structure of the enamel, as laid down, but upon protein deposits or chemical changes acquired after eruption of the teeth. A related type of investigation made by Staz (1282) who found that the teeth of the caries-resistant primitive Bantu were no more resistant to the decalcification process than the much more caries-susceptible urban Bantu and Europeans. Klinger (1064) and Volker (563) found no difference in solubility of enamel of carious and non-carious teeth.

The increasing interest in enzymes and co-enzymes in many fields has been reflected in caries research. It offers a promising field of investigation. One of the first to receive attention was amylase, while still identified by its earlier name of ptyalin. Pickerill (969) claimed a difference between mouths of the caries-resistant Maori and caries-







susceptible Europeans. Bergeim (531) found no correlation between amylase and caries. Dean (1191) and McClure (1112) found no effect from fluoride waters on amylase activity. Pratt (900) found wide individual variations in amylolytic activity of saliva. Turner (1343) thinks there is a correlation between active caries and a rapid hydrolyzing time. He, as well as Volker (1619), have carried out extensive investigations on amylase.

Fosdick (1146), Shafer (1147), Rae (728), Lura (1806) and Dentay (2212) have made recent investigations on phosphatase.

Hine (841) has dealt with the presence of urease producing bacteria and dental caries activity, as shown by presence of acidogenic and aciduric bacteria.

The carbohydrate degradation process in the mouth has received considerable attention from Fosdick (689, 540, 1274, 547, 1599), Burrill (520), Dreizen (1342, 1325), Calandra (1598) and Stephan (1819). The possible link up here with members of the vitamin B complex and the amino acids are only two phases of the carbohydrate degradation process problem, which seems to offer a promising field for further investigation.

The observations of such investigators as Smith (1541), Day (545), Dean (1114, 1116), Hodge (572), Deatherage (553, 557), Weaver (614) and others have established the fact that there is a decreased caries incidence in children born and reared in fluoride areas. Here again the data is chiefly concerned with caries in childhood and very few statistics are available concerning older age groups.

The fluorides are widely distributed in nature. Hill (2270) states that approximately 4.3% of the population of the U.S. use communal waters containing 0.5%, or more, fluorides. De Eds (1237), Lawrenz (571), McClure (609) and others have shown the very wide distribution of fluorides in food, particularly in tea, sea foods, some soups and some widely used infant foods. Sognnaes (721) appears to be the only person to have reported evidences of mottled enamel in a non-fluoride area, where the only possible source of fluorides was from the food. It has been assumed that the caries inhibiting effect of fluorides is exerted only when the fluorides are contained in the drinking water. It is difficult to establish this point positively, since no method has yet been devised for the trial of a fluoride-free diet.

The observation of the caries-inhibiting property of fluorides has resulted in two main fields of investigation: (1) The addition of fluorides to communal waters, and (2) Topical application of fluorides. The value and role of the addition of fluorides to communal waters is largely subject to study by public health authorities and is practically limited to people living in communities served by a central water supply system. It will take some years before its value can be assessed. The value of topical application of fluorides is a problem which lends itself to immediate investigation.

Topical application of fluorides has received wide attention in recent years. The reports of Knutson (578, 1377) and others have led to claims of approximately 40% caries reduction. This figure arrived at on the basis of the difference in the number of new lesions developing







on treated and control quadrants of the same mouth. While this statement is basically correct statistically, it is a question whether it has not given rise to a misconception of the value of the treatment. Krasnow (2014) points out that this idea that a few topical applications of fluorides results in a general impression that 40 teeth out of every 100 are saved from decay. This magnifies the basic rate ten-fold. If juxtaposed by statistics of rate per cent, relative percentages do not convey completeness of connotation. This is especially so when rates are comparatively small. Thus, in one experiment where 7869 teeth were treated, 5.1% developed caries while in 7849 untreated teeth 8.8% developed caries, therefore the percentages of teeth without caries were 94.9 and 91.2%, or an actual improved result of 3.7%. He considers that this more accurately assesses the value of the treatment. Stones (2248) has recently reported on work in England, which has been more carefully done than much of the previously reported work on this subject and he found no significant caries reducing effect by topical application of fluorides in his experiments.

If it is assumed that topical applications of fluorides have some caries reducing effect then it is interesting to try to determine the reasons for the beneficial effect. There have been four main theories advanced: (1) An inhibiting effect on acid production in the mouth, (2) Anti-bacterial action, (3) Anti-enzyme action, (4) Effect on tooth solubility. These, of course are not necessarily separate and distinct, but merely divided in this way for purposes of discussion. There is no satisfactory proof that fluorides exert their effect in the mouth by any of these methods. Volker (1538) found that rate of multiplication of mouth organisms was not affected until fluorine concentrations of 250-500 p.p.m. reached, which is far beyond any concentration in human mouth. Arnold (1115) found no indication that increased fluoride content of water supply of an area had any effect on *L. acidophilus* counts, although others have made such claims. Goodman (1374) concluded that a 1:1000 solution of sodium fluoride has practically no inhibitory effect on bacteria. McClure (1112) obtained no effect from fluorides on amylolytic enzymes at quantities even greater than those which would be used in the mouth. Saliva from children of a 1.8 p.p.m. area showed no effect on amylolytic enzymes. On the contrary, Hodge (1611) states that, as an enzyme poison, fluorine blocks the formation of lactic acid by preventing transformation of glycerol phosphoric acid into phosphopyruvic acid. It also inhibits phosphatase. Volker (1051), Goodman (1374), Bibby, (1375) and Muhler (1346, 1850) found that when powdered enamel was treated with various fluoride salts it showed decreased solubility. Phillips (1884) obtained somewhat similar results on cleaned, dry, polished enamel sections. It is a question how far these in vitro results apply to in vivo conditions and there is reason to believe that the freshly exposed enamel surface of these tests reacts with the fluorine in a different manner from that of the enamel surface in the mouth. No reference has been found to any experiment where a comparison was made on the effect on solubility of fluoride treated and untreated teeth in an animal. While the theories for the method of action of fluorides in the mouth are inconclusive at this time, it is







still an interesting field and certainly the relation of the fluoride-ion to the carbohydrate degradation process would seem worthy of further study.

If topical application of fluorides is of benefit, there must be some explanation of the reason for the retention of the fluoride-ion in the mouth for extended periods. The general explanation has been that it in some way becomes fixed to the surface of the tooth. Schour (777) has shown the effect of fluorides on calcifying tooth tissue. Hodge (1230) thinks that since the line plotted by the amount of fluorine taken up by enamel is similar to the Freundlich adsorption isotherm then this surface union is due to adsorption. Gerould (526) believes that it is the result of a double decomposition. Whatever the actual mechanism is, it would at least appear that the tooth surface must be clean. This has not received sufficient attention in suggested techniques of topical application. Arnold (578), Bibby (1324) and others have stressed the need for preliminary prophylaxis. A number of workers have pointed out the better results on smooth surfaces, which would be much more readily cleaned and dried. While many workers stress the need for a preliminary prophylaxis, no reference has been found to suggest that careful prophylaxis should be carried out each time, just previous to the fluoride application. This lack of thoroughness in cleaning the tooth surface before each application appears to be scientifically unsound. The reported differences in the results obtained by use of different fluoride salts is puzzling and unexplained.

The topical application of fluorides has received so much publicity that the question naturally arises as to just how much value it has in caries prophylaxis. If properly done, it is a time-consuming procedure, since as indicated above there are scientific reasons why an immediately preceding thorough cleaning and drying of the teeth is necessary. This definitely limits effectiveness of the treatment on approximating tooth surfaces. Treatments must be repeated a number of times, in order that each group of new teeth will be treated within a short period of their eruption into the mouth. It is always dangerous to prophesy, but when the above factors are considered, it certainly seems doubtful whether the treatment will have any wide field of usefulness in the future.

This is not to say that fluorides should be dropped from research projects, there are still too many unsolved phases of the role of fluorides in caries. There has been a marked drop in the number of reports on fluorine investigations. This is unfortunate. There is unquestionable data on the decreased incidence of caries, at least in young people, in fluoride areas. There is a possibility, but not acceptable proof, that young people who move into fluoride areas before they are six, receive some benefit. Some information on this phase may be obtained shortly from the experimental areas where fluorides are being added to the communal water. Armstrong (354) and Hodge (1230) found that there were differences in the amount of fluorine in carious and non-carious teeth. These differences were very slight. McClure (1863) questions the value of this data and in the light of our inadequate knowledge of the possible variation in amounts of fluorine in teeth of dif-







ferent persons and probably in different teeth of the same person it is questionable whether these differences are significant. The story of fluorine and caries is thus so involved, so full of apparent contradictions, that there is a feeling that something vital may have been missed and that further research, perhaps along new lines, might prove valuable.

The most active period of the search for a bacterial cause of dental caries has been largely influenced by medical research in diseases which proved to be the result of a particular pathogenic organism. Dental caries is signalized by the destruction of tooth material and as such is quite dissimilar to these diseases. The result has been that there has been over emphasis on the search for a single causative organism. It would appear more likely that dental caries may be the result of more than one organism, which may be directly or indirectly involved in production of the lesions. The observed progress of the lesion points strongly to the possibility that the tissue destruction in dental caries could be the result of the action of a number of different organisms. The multiplicity of organisms present in the mouth, possible varying rates of tooth tissue destruction, ranging all the way from periods of complete inactivity of the process to periods of active destruction and the slow development of the lesion in the pre-clinical stage offer very great difficulties in this investigation. Increased concentration on the metabolic requirements of groups of organisms, such as the acid-forming and proteolytic, might prove valuable.

There is an extensive literature and a multitude of opinions on the relation of microorganisms to caries. Howe (1444) thought that the difference between carious and immune mouths, as indicated by acid production, was chiefly in the predominance of organisms capable of producing larger quantities of acid in mouths where caries was present. Gies (294) stressed importance of streptococci and filamentous organisms, Hadley (1127), in 1924, stated that early investigations were mostly concerned with the oral flora, rather than with those of the actual lesion, since it was not believed that a specific flora for caries existed. This idea soon changed and, in 1915, Kligler (1427) thought that there was a specific flora for caries. Bunting (245) referred to the difficulty of determining the degree of susceptibility of a mouth at any given time. Rodriguez (184) thought that occurrence and rapidity of development of the carious process bears a direct relationship to the number of *B. acidophilus* organisms in the mouth. Bunting (245, 963, 1007) tried various antiseptics in an attempt to control *B. acidophilus* and caries. He later (1748, 1408) concluded that a minimum of sugars and pastries exerted the greatest inhibitive effect. Enright (77), Bunting (245, 963), McIntosh (227) and Jay (237) all used a strongly acid broth to prevent growth of all except aciduric types of organisms. The use of a broth adjusted to pH 5 practically insures the growth of a pure culture of *Lactobacillus*. None of these show sufficient reason to justify a choice of procedure which immediately removed from consideration other acid-producing bacteria of the mouth. Bibby (42) thought that the action of acid-producing organisms alone will not remove the entire enamel structure and that there must also be certain proteolytic agencies.







Hadley (15) gave her technic for the quantitative determination of *B. acidophilus* in saliva. Bertels (1658) referred to the necessity for considering the symbiotic, metabiotic and antibiotic associations of oral microorganisms. Boyd (17) thought that since effect of bacteria in production of caries is directly related to formation of acid through fermentation that attention should not be limited to *B. acidophilus*, or any other single type of organism. Bibby (1220) states that some workers claimed to have established a close correlation between lactobacilli and caries, others question the validity of this and still others point out that the organism may appear or proliferate as a result of the formation of cavities. The predominance of lactobacilli in carious cavities does not prove per se that these organisms produced the cavities but only that conditions in them favoured growth of these organisms. He criticizes proponents of the theory of a specific bacterial cause for caries because of their failure to study the process in its initial stages and made little or no effort to discover bacterial causes, other than the presence of lactobacilli. If highly selective media had been used for other organisms, which might be associated with caries, it is possible that interesting correlations might have been developed. Anderson (313) found a closer relationship between streptococci and caries than between *B. acidophilus* and caries. Bibby (429) found no constancy in the percentage distribution of different morphological types of organisms in the cavities studied. Rosebury (371) in his extensive caries review, strongly supports the *B. acidophilus* school, even to the point that workers who did not come to this conclusion must have used faulty techniques in their studies. Gins (466) criticized lack of study of the anaerobic types. Bradel (916) used a selective culture method to determine incidence of certain specific organisms in caries-active and caries-free mouths and found no definite correlation between *B. acidophilus* and caries. Bibby (631) thinks that accumulations of food stuffs in the mouth may upset the normal regulatory mechanisms and where these accumulations are carbohydrates, the types of organisms which thrive in an acid medium will proliferate. Bibby (1823) thinks that cavities provide environments where the lactobacilli are protected from the saliva and other organisms are put at a disadvantage, thus the lactobacilli may be the result of and not the cause of caries. Information on the relative importance of different types of organisms in caries must await development of cultural methods equally favorable to all types of bacteria. The demonstration that any organism is more closely related to caries than any other is not proof of a causal relationship, as this may be due simply to better growth conditions provided by the cavity. There is as yet no reliable information on manner of destruction of enamel fraction of dentin, so that for the present consideration must be limited to a consideration of action of bacteria which act on calcified portions of tooth, which depends on ability of organisms to produce acid. Blayney (687), over a 5-year period, cultured areas before and after appearance of cavities. They found a definite increase in incidence of lactobacilli as cavity increased in size, but did not draw any conclusions as to exact relationship of the lactobacilli to caries.







The role of the organism variously termed *B. acidophilus*, *Lactobacillus*, etc, has received the greatest amount of attention in caries literature. There is acceptable proof of the association of this organism with caries. There is not proof that it is the cause of caries. It would appear that counts of this organism are valuable indicators as to whether or not conditions in a mouth are favorable, or otherwise, for the growth of this type of acid-producing organisms. This index should only be used as an indicator of this nature and not in the belief that it furnishes a positive index of the invasion of a specific caries-producing organism.

The ability of mouth organisms to produce acid has been studied by a number of workers. Hansen (419) found that the combination of organisms which produced the greatest amount of acid from cooked starch was *B. subtilis*, *B. acidophilus* and *Saccaromyces cerevisiae*. Bibby (1893) found that streptococci were consistently more numerous in mouths with active caries and that they produced acid more rapidly, but after 24 hours the titratable acidity produced by streptococci was somewhat less. Harrison (1420) stated that microorganisms must be able not only to produce acids, but also to grow and metabolize in an acid environment in order to contribute to decalcification of tooth substance. Fosdick (696) found that acid formation is much more rapid in the presence of yeast and *L. acidophilus* than with either organism separately. Stephan (1349) questioned whether the study of microbic cells at the low concentrations of traditional bacteriological methods can give an accurate picture of what these microorganisms can do at the high concentrations of the bacterial plaque. He found that at concentrations similar to those obtaining in the mouth that, in general, the monosaccharides produced the most rapid pH changes, with sucrose and maltose next, while lactose and soluble starch showed the slowest pH changes.

A number of workers have attempted to reduce bacterial counts in the mouth by the use of germicides. They obtained a rapid, but temporary reduction. Hine (841) found absence of *L. acidophilus* in mouths containing urease producing bacteria. Dreizen (1785) found evidence that the end products of tissue break-down exerted an inhibiting effect on acidogenic bacteria. Kesel (1859) thinks that an agent introduced in the mouth which would depress certain types of organism, while stimulating others, might have a quite lasting effect. Thinks that the oral flora may be susceptible to changes in the oral environment which may be too insignificant to be detected by chemical analysis and that the microbiologic assay method holds out some interesting possibilities in this connection. Dreizen (2255) has quite recently reported on the prolonged bacteriocidal activity of some of the nitrofurans compounds. It is difficult to see the point of some of these experiments at this time, except in so far as they add to our knowledge of the metabolic requirements of such groups as the acid forming microorganisms. The use of the antibiotics, as penicillin, is open to criticism. The objective to be gained is not clear cut and the indiscriminate use of such agents for this purpose might have rather serious results for the patient. Wallace (1816) made a number of comments which should be in the minds of all who are







engaged in caries research. He pointed out that since caries is not a fatal disease and people can live without their teeth that it is not justifiable to use either drastic methods or drastic materials to control caries. In general, caries control method should be harmless, the simplicity of the method is important and it should be acceptable to the patient. He criticizes the use of drugs which are without value and which give an unwarranted feeling of security. The mechanism of action of drugs should be understood before general use is recommended.

The nutritive requirements of bacteria is a comparatively recent field of investigation, which holds promise. Hill (1076), Fossdick (1131), Kniesner (812) Weisberger (1285, 1301, 1322) and Orland (1337) have contributed to the literature on the nutritive requirements of lactobacilli.

The possible relationship of some filamentous organisms to caries has attracted recent attention by Bibby (1734, 1823), and Bartels (840). Hurst (1780) has carried out work on an *Actinomyces* type of organism and the work of the group with which he is associated is interesting and may well be significant. It will be followed with interest.

The rat has been the most popular experimental animal for caries research. The species most often used was the white rat, although in recent years there has been a tendency on the part of some investigators to use the cotton rat. The early experiments were almost entirely dietary and were started at a time when the "adequate diet" theory for building sound, caries-resistant teeth was at its height. It was soon noted that different workers, using diets of similar composition, were obtaining quite diverse results. Hoppert (44) noted these differences in 1932 and decided that the variable factor was the particle size and hardness of the rice and grain diets generally used. It was established that the usual caries lesion in the white rat developed following cusp fracture. This is not a factor in human caries. It also was shown later that larger dietary doses of fluorine were required to obtain similar effects on teeth of rat and of man. These are a type of limiting factors which should be kept in mind in animal experiments and show that care must be taken in assessing the results obtained in animal experiments as directly applicable to humans. Bodecker (152) and Rosebury (1895) state that the lesion in rat teeth begins in the enamel, rather than in the dentin. Bibby (16) comments on the high incidence of caries in lower teeth of rat, as compared with uppers, which is the reverse of that in man. Klein (91), Shelling (12), Smith (459) and Braunschneider (1852) have all referred to a relationship between age of rat and caries. McClure (590), Hunt (830, 904, 1837), Schweigert (1604) have shown a variation in caries incidence in different strains of animals. These findings have shown the necessity of using litter mates in test and control groups.

A number of dietary experiments with different types of carbohydrates have been carried out on rats since the identification of the presence of the "particle size" factor. Lilly (1640) found fewer cavities when commercial casein substituted for whole milk powder. Rosebury (482) found a decreased incidence when corn oil added to the diet.







They believed that the free oil was more valuable in this respect than natural oil bound in the corn. Box (1095) thought that the greater dispersion of the natural oil in finely ground grain was more effective than the lesser dispersion in larger particles. Hodge (1917) reported more extensive, but not more numerous, lesions in animals on a diet containing 50% sucrose. Schweigert (1535) found no appreciable difference between coarse and fine dextrin rations. Where as little as 1/6 of the dextrin was replaced by sucrose there was a large increase in caries. They believed this was due to the fact that dextrin is not readily fermented by acid-forming organisms of the mouth. Schweigert (1607) found a sharp decrease when sucrose replaced by starch. Starch and dextrin were much less cariogenic than the fermentable carbohydrates used. Where proteins and fats were increased at expense of carbohydrates there was decreased caries incidence. Schweigert (1606) obtained almost complete protection from caries in a diet containing 20 parts of lard, although it also contained 22 parts sucrose. Think that this is not entirely due to replacement of part of the sucrose by fat and proteins, but that these favourable effects might be associated with a change in the bacterial flora resultant from use of a high protein diet and/or formation of a protective oily coating for the teeth. Anderson (1838) found that liquid whole milk affords considerable protection from caries in the cotton rat, even when associated with fairly high levels of fermentable sugar in the diet. He, and his colleagues, point out that the fat content is in a relatively free form in liquid milk. Howell (1849) was unable to find any protective effect from presence of oxalates in the diet. Sognnaes (1998) reported that use of a purified diet in animals, where molars had been extracted to prevent cusp fractures, produced lesions which more closely resembled those of man. Much of this work has been carried out in the past few years.

The rat has also been widely used in fluoride experiments. The amount of fluorides used in dietary experiments is much larger than would be received by man. Volker (1538) concluded that intake of fluorides necessary to produce changes in caries incidence in rats is far in excess of that which modifies human caries. This may be the result of greater fluoride resistance in the rat. Shaw (1605) found that the level of fluorides necessary to inhibit caries in the cotton rat was similar to that required for the white rat, even though the method of producing caries in the two species is quite dissimilar. Hodge (1611) gives an extended review on rat experiments with fluorides. Arnim (1731), Miller (576) and Cox (493) described methods for identifying and recording rat lesions. Hodge (1611) found that while fluoride administration during tooth development is shown by mottling and increased fluorine content of teeth, this is not sufficient to provide a high level of resistance when the rat is exposed to a caries-producing diet.

The oral microorganisms of the rat have been studied by a number of workers, including Rosebury (79), Klein (81), Johnston (29), Greenberg (1138), Harrison (577, 1420, 1421, 1422) and Parsons (2084). McClure (1335) and Webman (2250) reported a decrease in caries incidence when penicillin administered. Wakemann (1811) found that numbers of







anaerobic organisms were proportionately higher in deep fissure caries of the rat. He later (1877) was impressed by the finding that proteolytic enterococci were more numerous than lactobacilli in cultures isolated from ground caries lesions of rat molar.

The close physical relation of the saliva to the caries lesion has long been noted and many papers have appeared on the saliva. The pattern of these investigations until quite recently was the attempt to find some correlation between the buffering capacity of the saliva and acid formation at the site of the caries lesion. There are long series of reports on pH of the saliva, buffering capacity, rate of flow, composition and, more particularly, the inorganic composition. Much of this work seems to have been based on the idea that the saliva was in free contact with the developing lesion. There was no general realization that different physical conditions may operate in different cavities. The physical problems of the osmotic membrane formed by the plaque and the buffering ability of the saliva must be considered in terms of this limiting membrane. The recent work on the ammonium-ion is of particular interest since it has greater ability to pass through an osmotic membrane than most of the salts of the saliva previously investigated. The tiny opening of some pit and fissure cavities which, when enlarged by instruments, may show extensive destruction of the inner part of the tooth. Here also, quite apart from any question of a plaque, there must be quite inadequate interchange with the saliva. Then, at the other extreme, the cavity with a large opening and the possibility of freer exchange with the saliva.

The more recent investigations of saliva have been along different lines. Dreizen (1865) made in vitro studies of the effect of the putrefaction products of proteins on the growth of aciduric and acidogenic organisms. Grove (146) suggested that the immune factor in saliva is an adequate supply of ammonia. White (1570) found no relation between ammonia of saliva and caries. Youngburg (119) found that salivary ammonia nitrogen values for normal individuals were between 1.28 and 13.66 milligrams per 100 cc of saliva. Stephan (836) found average concentration of urea in resting saliva is about 0.02%. He thinks that the extent to which different concentrations of urea in saliva may act to restrict occurrence of caries would be extremely difficult to determine, due both to the number of variable systemic factors which control the concentration of urea in saliva and also to the many variable local factors which influence the flow of saliva to different tooth surfaces. Kesel (1416) thinks that development of ammonia nitrogen in the oral cavity may explain the caries resistance of some persons. Kesel (1636) found that ammonia is not produced in salivary cultures, if glucose is present in the mixture. He carried out a number of tests to determine effect of whole saliva on deamination of the various amino acids. He claimed to find a difference between caries-resistant and caries-susceptible persons in this respect. Weisberger (1341) found that the presence of glucose in saliva favors the process of proteolysis, rather than deamination. Kirch (1597) found no relation between quantities of amino acids present in saliva and caries activity. These recent trends in saliva research would appear more promising and more in harmony with our knowledge of the caries lesion than most of the earlier investigations on the saliva.







This summary of caries research has attempted to give a short review of the main trends of investigation in the hope that it might make some distinction between avenues of effort which do not appear promising, at this time, and to help further investigation in fields which hold promise for a measure of control of dental caries.

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2273. Hurst, V., P. Mullett, H.E. Frisbie, J. Nuckolls, M. Marshall. A progress report on the bacteriological aspect of the caries research project, College of Dentistry, Univ. of California. J.D.Res. 28: 430-432, 1949.



Mar 2-3, 1950

T.J. Rae (A. Legg).

54

R.A. Kuegel (Boc.)

ag. Mitley

Doreen Smith.

hermat. leather

D 5, watt.



# APPENDIX "U"

## National Research Council

### Associate Committee on Dental Research

#### Grantees or Assistants invited to report personally

|                      | <u>Reporting</u>   | <u>Grantee</u>     | <u>Project No.</u> |
|----------------------|--------------------|--------------------|--------------------|
| <u>3rd Meeting</u>   |                    |                    |                    |
| Feb. 18, 1946        | Dr. G. H. Lucas    | Dr. Lucas          | DR 2               |
|                      | Dr. J. J. Rae      | Dr. Rae            | DR 1               |
| <u>4th Meeting</u>   |                    |                    |                    |
| Oct. 22, 1946        | Mr. D.R. Christie) | Dr. Westman        | DR 5               |
|                      | Dr. A.E. Westman ) |                    |                    |
|                      | Dr. G.H. Lucas     | Dr. Lucas          | DR 2               |
| <u>5th Meeting</u>   |                    |                    |                    |
| Feb. 25, 1947        | Dr. J.S.Bagnall    | Dr. Bagnall        | DR 7               |
| <u>6th Meeting</u>   |                    |                    |                    |
| Oct. 21, 1947        | Mr. D.R.Christie   | Dr. Westman        | DR 5               |
|                      | Mr. D.R.Christie   | Dr. Westman        | DR 12              |
|                      | Dr. G.M.MacDonald  | Dr. MacDonald      | DR 10              |
|                      | Dr. C.H.Moses      | Dr. Godfrey        | DR 11              |
| <u>7th Meeting</u>   |                    |                    |                    |
| Mar. 2, 1948         | Dr. J.S.Bagnall    | Dr. Bagnall        | DR 7               |
|                      | Dr. M.D. Smith     | Dr. Smith          | DR 13              |
|                      | Dr. M.D. Smith     | Dr. Smith          | DR 14              |
|                      | Dr. N.F. Walker    | Dr. N.F.Walker     | DR 15              |
| <u>8th Meeting</u>   |                    |                    |                    |
| Oct. 26, 1948        | Dr. G.H.W.Lucas    | Dr. Lucas          | DR 2               |
|                      | Dr. G.M.MacDonald  | Dr. MacDonald      | DR 10              |
|                      | Dr. C.H.Moses      | Dr. R.J.Godfrey    | DR 11              |
| <u>9th Meeting</u>   |                    |                    |                    |
| Mar. 3 and 4, 1949   | Dr. R.L.Hugill     | Dr. H.K.Box        | DR 18              |
|                      | Dr. O.J. Walker    | Walker and MacLean | DR 9               |
|                      | Dr. M.D. Smith     | Dr. Smith          | DR 13              |
|                      | Dr. M.D. Smith     | Dr. Smith          | DR 14              |
|                      | Dr. J. Thébaud     | Dr. Thébaud        | DR 20              |
| <u>10th Meeting</u>  |                    |                    |                    |
| Oct. 17 and 18, 1949 | Dr. G.H. Lucas     | Dr. Lucas          | DR 2               |
|                      | Dr. J.S. Bagnall   | Dr. Bagnall        | DR 7               |
|                      | Mr. D.R.Christie   | Dr. Westman        | DR 12              |
|                      | Mr. D.R.Christie   | Dr. Westman        | DR 21              |
|                      | Dr. W.L.Linghorne  | Dr. Best           | DR 22              |
|                      | Dr. L.F.Bélanger   | Dr. Leblond        | DR 23              |







"U-2"

| <u>Project</u> | <u>Reported by Grantee or Assistant</u> |
|----------------|-----------------------------------------|
| DR 1           | 1 (member of Committee)                 |
| DR 2           | 4                                       |
| DR 3           | 1 (member of Committee)                 |
| DR 4           | 1 (member of Committee)                 |
| DR 5           | 2                                       |
| DR 6           | 1 (member of Committee)                 |
| DR 7           | 3                                       |
| DR 8           | 0                                       |
| DR 9           | 1 (member of Committee)                 |
| DR 10          | 2                                       |
| DR 11          | 2                                       |
| DR 12          | 2                                       |
| DR 13          | 2                                       |
| DR 14          | 2                                       |
| DR 15          | 1                                       |
| DR 16          | 1 ( member of Committee)                |
| DR 17          | 0                                       |
| DR 18          | 1 ( member of Committee)                |
| DR 19          | 0                                       |
| DR 20          | 1                                       |
| DR 21          | 1                                       |
| DR 22          | 1                                       |
| DR 23          | 1                                       |
| DR 24          | 0                                       |
| DR 25          | 1                                       |
| DR 26          | 0                                       |







## APPENDIX "V"

### Policy of the Associate Committee on Dental Research regarding Publications and Reports

(As adopted by the Committee)  
Min.37:10th Meeting, 18/10/49

---

1. Grantees in their periodic reports shall inform the Committee of the portions of their work which are to be submitted to some Journal or publisher for publication.
  2. Acknowledgment of assistance by the Associate Committee on Dental Research shall appear on the publication. Whenever consistent with the policy of the journal or publication the acknowledgment shall take the form of a footnote saying: "Assisted by a grant from the Associate Committee on Dental Research of the National Research Council of Canada".
  3. When such publications appear the grantee shall inform the Secretary of the Associate Committee on Dental Research giving the author or authors, title and reference.
  4. The Secretary of the Associate Committee on Dental Research when notified of a publication on work assisted by the Associate Committee on Dental Research shall assign a number to the publication and shall list this number together with the author, title and reference as an appendix to the minutes of the meeting of the Associate Committee on Dental Research.
  5. The grantee shall supply, when they become available, twenty-five reprints of each publication for the use of the National Research Council.
  6. Reprints of each publication shall be available to each member of the Associate Committee on Dental Research.
-







## INITIAL DISTRIBUTION

### (i) To Members of the Committee

#### Associate Committee on Dental Research

|                                                                               | <u>Term to Expire</u> |
|-------------------------------------------------------------------------------|-----------------------|
| *1. Dr. R. G. Ellis, <u>Chairman</u>                                          | 31 March, 1951        |
| 2. President C. J. Mackenzie (ex officio)                                     | --                    |
| <u>Rep. Dental Profession</u>                                                 |                       |
| 3. Dr. H. K. Box                                                              | 31 March, 1952        |
| *4. Dr. E. Charron                                                            | 31 March, 1951        |
| 5. Dr. M. H. Garvin                                                           | 31 March, 1950        |
| 6. Dr. H. R. MacLean                                                          | 31 March, 1950        |
| 7. Dr. R. H. McDougall                                                        | 31 March, 1952        |
| <u>Rep. Royal Canadian Dental Corps</u>                                       |                       |
| *8. Col. E. M. Wansbrough (ex officio)                                        | --                    |
| <u>Rep. Division of Dental Health</u><br><u>Dept. Nat. Health and Welfare</u> |                       |
| 9. Dr. H. K. Brown                                                            |                       |
| <u>Rep. Basic and Med. Sciences</u>                                           |                       |
| 10. Dr. C. H. Best                                                            | 31 March, 1950        |
| 11. Dr. J. B. Collip                                                          | 31 March, 1952        |
| 12. Dr. O. W. Ellis                                                           | 31 March, 1951        |
| 13. Dr. J.K.W. Ferguson                                                       | 31 March, 1951        |
| 14. Dr. A. W. Ham                                                             | 31 March, 1952        |
| 15. Dr. J. J. Rae                                                             | 31 March, 1950        |
| 16. Dr. C. B. Stewart                                                         | 31 March, 1951        |
| *17. Dr. D. L. Thomson                                                        | 31 March, 1950        |
| *18. Mr. S. J. Cook, <u>Secretary</u>                                         | --                    |

### (ii) To Other Persons:

19-21 Deans of Dental Schools (who are not members of the Committee)

- 19. Alberta - Dr. W. S. Hamilton
- 20. Dalhousie - Dr. J. Stanley Bagnall
- 21. McGill - Dr. D. Prescott Mowry

- 22. Master Copy (General Secretary)
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25-30 Reserve.

\* Members of the Executive















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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
OF THE  
EIGHTH MEETING  
OF THE  
EXECUTIVE  
OF THE  
ASSOCIATE COMMITTEE  
ON DENTAL RESEARCH

OTTAWA

2 MARCH, 1950







Proceedings of the Eighth Meeting of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Room 247 Chateau Laurier, 7:30 p.m., 2 March, 1950.

1. Attendance

Dr. R. G. Ellis, Chairman  
Dr. E. Charron  
Dr. D. L. Thomson  
Col. E. M. Wansbrough

Mr. S. J. Cook, Secretary

Other members of the Committee who were present for part of the meeting included:

Dr. H. R. MacLean  
Dr. R. H. McDougall  
Dr. O. W. Ellis

2. Consideration of Applications for Grants

THE CHAIRMAN reviewed the financial position of the Committee pointing out that there is a substantial balance remaining to the Committee's credit in 1949-50 funds and that more applications had been received for 1950-51 grants than the Committee had funds to meet. On consideration it was decided that if any applications for renewals of grants included equipment which could be purchased out of this year's funds, recommendations to this effect would be made.

Recommendations were made regarding applications for grants as tabulated hereunder:

| <u>No. and Name</u>                | <u>Applications<br/>for<br/>1950-51</u><br>\$ | <u>To be bought<br/>out of<br/>1949-50 funds</u><br>\$ | <u>Amount recom-<br/>mended for<br/>1950-51</u><br>\$ |
|------------------------------------|-----------------------------------------------|--------------------------------------------------------|-------------------------------------------------------|
|                                    | <u>RENEWALS</u>                               |                                                        |                                                       |
| DR 1 - Dr. J. J. Rae               | 1500                                          | 550                                                    | 950                                                   |
| DR 2 - Dr. G. H. W. Lucas          | 1550                                          |                                                        | 1550                                                  |
| DR 12) Dr. A. E. R. Westman<br>25) | 4000                                          |                                                        | 3000                                                  |
| DR 13 - Dr. M. D. Smith            | 1475                                          |                                                        | 1475                                                  |







RENEWALS (cont'd)

| <u>No. and Name</u>       | <u>Applications<br/>for<br/>1950-51<br/>\$</u> | <u>To be bought<br/>out of<br/>1949-50 funds<br/>\$</u> | <u>Amount recom-<br/>mended for<br/>1950-51<br/>\$</u> |
|---------------------------|------------------------------------------------|---------------------------------------------------------|--------------------------------------------------------|
| DR 15 - Dr. N.F. Walker   | 800                                            |                                                         | 800                                                    |
| DR 18 - Dr. H. K. Box     | 2120                                           |                                                         | -                                                      |
| DR 22 - Dr. C.H. Best     | 2600                                           |                                                         | 2600                                                   |
| Dr. C.H. Best             | 1500                                           | 130                                                     | 1200                                                   |
| DR 23 - Dr. C.P. Leblond  | 2800                                           | 400                                                     | 2400                                                   |
| DR 24 - Dr.C.H.M.Williams | 1735                                           |                                                         | 1735                                                   |
| DR 25 - Dr.A.E.R.Westman  | (See DR 12)                                    |                                                         |                                                        |
| DR 26 - Dr. R.E. Moyers   | 150                                            |                                                         | 150                                                    |
| DR 27 - Dr. J.W.Abbiss    | 300                                            |                                                         | 200                                                    |
| and<br>Dr. A.G. Nutlay    |                                                |                                                         |                                                        |
| DR 28 - Dr. O.J. Walker   | 1000                                           |                                                         | 1000                                                   |
| DR 29 - Dr. M.D. Smith    | 1500                                           |                                                         | -                                                      |
| DR 30 - Dr. N.F. Walker   | 2600                                           |                                                         | 1400                                                   |

NEW APPLICATIONS

|                           |                     |                   |                     |
|---------------------------|---------------------|-------------------|---------------------|
| DR 31 - Mr. G.J. Miller   |                     |                   |                     |
| and Dr. L.B. Jaques       | 1870                |                   | 1870                |
| DR 32 - Dr. A.W. Ham      | 1720                |                   | 900                 |
| DR 33 - Dr. H.A. Hunter   | 2500                |                   | 1500                |
| DR 34 - Dr. A. D'Iorio    | 3340                |                   | 2840                |
| DR 35 - Dr. J.B.MacDonald | <u>3150</u>         | <u>          </u> | <u>2000</u>         |
| <br>TOTAL                 | <br><u>\$38,210</u> | <br><u>\$1080</u> | <br><u>\$27,570</u> |







### 3. Membership of the Committee

The Executive recommends that all five retiring members of the Committee be invited to accept a further appointment of three years and that if they are agreeable they be nominated to the National Research Council for this additional term.

AGREED.

The Meeting adjourned at 10:30 P.M.







## INITIAL DISTRIBUTION

### (i) To Members of the Committee

#### Associate Committee on Dental Research

##### Term to Expire

- |                                       |                |
|---------------------------------------|----------------|
| * 1. Dr. R. G. Ellis, <u>Chairman</u> | 31 March, 1951 |
| 2. Dr. C. J. Mackenzie (ex officio)   | --             |

#### Rep. Dental Profession

- |                        |                |
|------------------------|----------------|
| 3. Dr. H. K. Box       | 31 March, 1952 |
| * 4. Dr. E. Charron    | 31 March, 1951 |
| 5. Dr. M. H. Garvin    | 31 March, 1953 |
| 6. Dr. H. R. MacLean   | 31 March, 1953 |
| 7. Dr. R. H. McDougall | 31 March, 1952 |

#### Rep. Royal Canadian Dental Corps

- |                                         |    |
|-----------------------------------------|----|
| * 8. Col. E. M. Wansbrough (ex officio) | -- |
|-----------------------------------------|----|

#### Rep. Division of Dental Health Dept. Nat. Health and Welfare

- |                    |    |
|--------------------|----|
| 9. Dr. H. K. Brown | -- |
|--------------------|----|

#### Rep. Basic and Med. Sciences

- |                                        |                |
|----------------------------------------|----------------|
| 10. Dr. C. H. Best                     | 31 March, 1953 |
| 11. Dr. J. B. Collip                   | 31 March, 1952 |
| 12. Dr. O. W. Ellis                    | 31 March, 1951 |
| 13. Dr. J.K.W. Ferguson                | 31 March, 1951 |
| 14. Dr. A. W. Ham                      | 31 March, 1952 |
| 15. Dr. J. J. Rae                      | 31 March, 1953 |
| * 16. Dr. D. L. Thomson                | 31 March, 1953 |
| * 17. Mr. S. J. Cook, <u>Secretary</u> | --             |

### (ii) To Other Persons:

18-20 Deans of Dental Schools (who are not members of the Committee)

- 18. Alberta - Dr. W. S. Hamilton
- 19. Dalhousie - Dr. J. S. Bagnall
- 20. McGill - Dr. D. Prescott Mowry

- 21. Master Copy (General-Secretary)
- 22. Board Room Copy
- 23. Library

24-30 Reserve.

\* Members of the Executive















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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
OF THE  
ELEVENTH MEETING  
OF THE  
ASSOCIATE COMMITTEE  
ON DENTAL RESEARCH

OTTAWA

2 - 3 MARCH, 1950







## TABLE OF CONTENTS

|                                                  | <u>Page</u> |
|--------------------------------------------------|-------------|
| 1. Attendance .....                              | 1           |
| 2. Chairman's Remarks .....                      | 1           |
| 3. Minutes of the 10th Meeting .....             | 2           |
| 4. Business Arising out of the Minutes .....     | 2           |
| 5. DR 1 - Dr. J. J. Rae .....                    | 4           |
| 6. DR 2 - Dr. G.H.W. Lucas .....                 | 4           |
| 7. DR 3 - Dr. H. K. Box .....                    | 4           |
| 8. DR 4 - Dr. H. K. Box .....                    | 4           |
| 9. DR 5 - Dr. R. L. Twible .....                 | 4           |
| 10. DR 6 - Col. E. M. Wansbrough .....           | 4           |
| 11. DR 7 - Dr. J. S. Bagnall .....               | 5           |
| 12. DR 8 - Dr. A.D.A. Mason .....                | 5           |
| 13. DR 9 - Drs. O.J. Walker and H.R. MacLean ... | 5           |
| 14. DR 10 - Dr. G. M. MacDonald .....            | 5           |
| 15. DR 11 - Dr. R. J. Godfrey .....              | 5           |
| 16. DR 12 - Dr. A.E.R. Westman .....             | 5           |
| 17. DR 13 - Dr. M. Doreen Smith .....            | 6           |
| 18. DR 14 - Dr. M. Doreen Smith .....            | 6           |
| 19. DR 15 - Dr. Norma Ford Walker .....          | 6           |
| 20. DR 16 - Dr. C. H. Best .....                 | 7           |
| 21. DR 17 - Dr. R. F. Shaner .....               | 7           |
| 22. DR 18 - Dr. H. K. Box .....                  | 7           |
| 23. DR 19 - Dr. J.K.W. Ferguson .....            | 7           |
| 24. DR 20 - Dr. Jules Thébaud .....              | 8           |







# TABLE OF CONTENTS (cont'd)

- 2 -

Page

|                                                 |    |
|-------------------------------------------------|----|
| 25. DR 21 - Dr. A.E.R. Westman .....            | 8  |
| 26. DR 22 - Dr. C. H. Best .....                | 8  |
| 27. DR 23 - Dr. C. P. Leblond .....             | 9  |
| 28. DR 24 - Dr. C.H. M. Williams .....          | 9  |
| 29. DR 25 - Dr. A.E.R. Westman .....            | 9  |
| 30. DR 26 - Dr. R. E. Moyers .....              | 9  |
| 31. DR 27 - Drs. J.W. Abbiss and A.G. Nutlay .. | 9  |
| 32. DR 28 - Dr. O.J. Walker .....               | 10 |
| 33. DR 29 - Dr. M. Doreen Smith .....           | 10 |
| 34. DR 30 - Dr. Norma Ford Walker .....         | 11 |
| 35. Publications .....                          | 11 |
| 36. Finances .....                              | 11 |
| 37. Attendance (Second Session).....            | 12 |
| 38. Chairman's Remarks (Second Session) .....   | 12 |
| 39. Applications for Grants-in-aid .....        | 13 |
| 40. Amounts of Grants 1950-51 .....             | 16 |
| 41. Fellowships .....                           | 16 |
| 42. Membership .....                            | 16 |
| 43. Air Travel .....                            | 17 |
| 44. New Business .....                          | 18 |
| 45. Date of Next Meeting .....                  | 18 |

## Appendices

Appendix "A" To study the effects of decreased barometric pressure on the oral tissues, DR 6.

Appendix "B" Assessment of local anaesthetics for small nerve-block, Dr. J.K.W. Ferguson, DR 19.







TABLE OF CONTENTS (cont'd)

- 3 -

Page

|              |                                                                                                                                                                                          |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Appendix "C" | An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry, Drs. O.J. Walker and H.R. MacLean, DR 9.                                   |
| Appendix "D" | Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon, Dr. C.P. Leblond, DR 23.                                                  |
| Appendix "E" | A histological study of tooth germ development both intra- and extra-alveolar with a survey of the pathological changes in the developing tooth, Drs. J.W.Abbiss and A.G. Nutlay, DR 27. |
| Appendix "F" | An investigation of the mechanism of repair of the supporting structures of the teeth, Dr. C. H. Best, DR 22.                                                                            |
| Appendix "G" | Chemical mechanisms in dental caries, Dr.J.J. Rae, DR 1.                                                                                                                                 |
| Appendix "H" | Cements for methacrylate inlays, Dr. A.E.R.Westman, DR 12.                                                                                                                               |
| Appendix "I" | The relining of lower dentures, Dr. A.E.R.Westman, DR 21.                                                                                                                                |
| Appendix "J" | Direct acrylic fillings, Dr. A.E.R.Westman, DR 25.                                                                                                                                       |
| Appendix "K" | The effects of hard and soft diets on the supporting structures of the teeth, Dr. C.H.M. Williams, DR 24.                                                                                |
| Appendix "L" | Improvement of subgingival curettage performed in the treatment of the periodontal pockets, Dr. J. Thébaud, DR 20.                                                                       |
| Appendix "M" | Biological absorption of fluorine, Dr.M.Doreen Smith, DR 29.                                                                                                                             |
| Appendix "N" | A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario, Dr. M.Doreen Smith, DR 13.                                    |
| Appendix "O" | Measurement of physiologic forces in the mouth, Dr. Robert E. Moyers, DR 26.                                                                                                             |
| Appendix "P" | Development of a photoelectric colorimetric method for determining small amounts of fluorides, Dr. O.J. Walker, DR 28.                                                                   |







## TABLE OF CONTENTS (cont'd)

- 4 -

- Appendix "Q" A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches, Dr. N.F. Walker, DR 15.
- Appendix "R" The study of the closure of space following premature loss of deciduous teeth, Dr. Norma Ford Walker, DR 30.
- Appendix "S" Experimental artificial production of dental caries in vitro, DR 18, Dr. H. K. Box.
- Appendix "T" Awards and expenditures to date under grants from the Associate Committee on Dental Research.
- Appendix "U" Associate Committee on Dental Research, Papers published.
- Appendix "V" Dr. Morrell's letter, Dr. Katzman's report on nitrous oxide anaesthetic gas machines and The Chairman's letter to Dr. Morrell.

### Initial Distribution

Dr. D. L. Thomson  
Dr. E. M. Wansbrough

Mr. S. J. Cook, Secretary

### By Invitation

Mr. C. T. Clegg  
Dr. R. A. Hugill  
Dr. A. G. Nutley  
Dr. M. Doreen Smith  
Dr. Norma Ford Walker  
Dr. D. G. Watt

Apologies for their absence were received from Dr. C. H. Best, Dr. H. K. Box, and Dr. J. W. Gillip. Dr. Gillip, however, wrote that he would attend the next day session.

### The Chairman's Remarks

The Chairman welcomed Dr. G. J. Mackenzie, President of the National Research Council and said that it was a pleasure to have the President at their meeting. He also welcomed







# NATIONAL RESEARCH COUNCIL

## Proceedings

### of the

### ELEVENTH MEETING

### of the

### ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,  
9:30 A.M., 2-3 March, 1950.

#### 1. Attendance

(1) Dr. R.G. Ellis, Chairman

Dr. H. K. Brown

Dr. E. Charron

Dr. O. W. Ellis

Dr. M. H. Garvin

Dr. A. W. Ham

Dr. C. J. Mackenzie

Dr. H. R. MacLean

Dr. R. H. McDougall

Dr. J. J. Rae

Dr. D. L. Thomson

Dr. E. M. Wansbrough

Mr. S. J. Cook, Secretary

#### By Invitation

Mr. C. T. Clegg

Dr. R. A. Hugill

Dr. A. G. Nutlay

Dr. M. Doreen Smith

Dr. Norma Ford Walker

Dr. D. G. Watt

Apologies for their absence were received from Dr. C. H. Best, Dr. H. K. Box, and Dr. J. B. Collip. Dr. Collip, however, wrote that he would attend the second day session.

#### 2. Chairman's Remarks

THE CHAIRMAN welcomed Dr. C. J. Mackenzie, President of the National Research Council and said that it was always an inspiration to have the President at their meeting. He also welcomed







Mr. Clegg who had come to report on DR 1, Dr. Walker who would report on DR 15 and 30, Dr. Smith to speak on DR 13 and 29, Dr. Hugill on DR 18, Dr. Watt on DR 24, and Dr. Nutlay on DR 27. He extended a cordial welcome to all these who had been working under grants from the Committee and invited them to take part in the discussions in the Meeting.

### 3. Minutes of the 10th Meeting

It was MOVED by Dr. Charron and SECONDED by Dr. O. W. Ellis:

That the Minutes of the 10th Meeting of the Committee, having been circulated, be taken as read and approved. CARRIED.

### 4. Business Arising out of the Minutes

#### (i) Bibliography on Caries Research (Min. 10, 10th Mtg.)

THE SECRETARY reported that in accordance with a decision taken by the Executive he had called for tenders for the printing of the complete report prepared by Dr. Bagnall on caries research. The lowest tender received was in the amount of \$3705. which with sales tax amounted to \$4001.40 for 100 copies. This included the retyping of the manuscript (single spacing) photographing and lithographing the copy, printing the book on 9 3/4 by 7" paper and binding in grey Buckram with the title printed in black ink on the spine only. Work is proceeding and about 100 pages have been retyped. The manuscript is being proof-read in the Secretary's office and checked by Dr. Bagnall before being ordered for print. It is expected the job will be completed within the fiscal year ended 31 March 1950. The cost of printing this book is being met out of the funds provided to the Committee for Fellowships since there were no applications for fellowships for 1949-50.

A shorter manuscript summarizing the results contained in the larger publication was prepared by Dr. Bagnall under the title "Trends in Caries Research". Tenders for the printing of this manuscript were called and the lowest tender received was \$355 for 5000 copies with cover. Copies of the printed booklet were distributed at the meeting and an additional copy is being mailed to each member with these proceedings. This booklet will be distributed to all members of the dental profession in Canada. It was suggested that the Secretary make the necessary arrangements with Dr. D.W. Gullett, 211 Huron St., Toronto, who is the Secretary of the Canadian Dental Association.

It was also suggested and AGREED that copies should be sent to the libraries of dental schools in Canada, the United States and Great Britain, to the provincial departments of health and to the professors of physiology and biochemistry in Canadian universities.







When the larger Bibliography is printed several copies will be sent to each of the Canadian dental schools as well as single copies to selected dental schools in the United States and Great Britain.

It was MOVED by Dr. McDougall and SECONDED by Dr. MacLean:

That the action of the Executive in arranging for the printing of the Bibliography on Dental Caries and the shorter booklet Trends in Caries Research be approved. CARRIED.

(ii) List of Committee Publications (Min. 37, 10th Mtg.)

THE SECRETARY said that a list of publications of the Associate Committee on Dental Research had been prepared and distributed to the membership with a request that additional items be forwarded regularly to the Secretary.

(iii) Anaesthetic Gas Machine (Min. 7, 9th Mtg.)

THE CHAIRMAN reported that a reply had been received from Dr. C.A. Morrell, Director, Food and Drug Divisions, Department of National Health and Welfare, enclosing copy of a report by Dr. J. Katzman on nitrous oxide anaesthetic gas machines. Dr. Morrell's letter, Dr. Katzman's report and the Chairman's letter to Dr. Morrell appear in

APPENDIX "V"

THE CHAIRMAN reported briefly on the history of the study of anaesthetic gas machines stating that the Committee had reported its findings to the Department of National Health and Welfare in the hope that that Department would take up the problem and deal with it. He had discussed the matter with Dr. Morrell personally and had pointed out that it was outside the scope of the Committee to make recommendations to the Department as to action which should be taken by it. He had suggested that the Canadian Dental Association be consulted and asked for recommendations.

DR. CHARRON suggested that the Canadian Medical Association also be invited to discuss this problem as it was of equal interest to them.

In a general discussion which followed it was the consensus of opinion that the Department of National Health and Welfare, having been given the problem by the Committee, should now proceed to consult the medical and dental profession if they needed any further advice as to possible official action designed to increase the accuracy of the gauge readings on anaesthetic gas machines.







Consideration of Reports from Grantees

5. DR 1 - Dr. J. J. Rae

"Investigation of the chemical mechanisms involved in decrease of dental caries by fluorides"

DR. RAE presented a summary and a full report which appear in  
APPENDIX "G"

MR. CLEGG reported to the Committee on section E, page G-6 of the report.

In reply to a question he said that the personnel referred to in Table 1, page G-3, were University of Toronto cadets and No. 1 Station R.C.A.F. and that they ranged in age from 18 to 26.

6. DR 2 - Dr. G.H.W. Lucas

" A study of alveolar oxygen tension and blood oxygen content during nitrous oxide-oxygen anaesthesia" - Project completed.

THE CHAIRMAN stated that a further application had been received from Dr. Lucas in accordance with a motion passed at a previous meeting of the Committee (Min. 5, 10th Mtg.). This would be dealt with under applications.

7. DR 3 - Dr. H. K. Box

" A study of micro-organisms found in periodontal pockets and dental caries as revealed by the electron microscope"

THE CHAIRMAN stated that this grant was to enable Dr. Box to make electron microscope photographs as material became available to him. He had drawn \$75. from his grant during the year for this purpose.

8. DR 4- Dr. H. K. Box

" Periodontal packs" - Project completed.

9. DR 5 - Dr. R. L. Twible

" Denture base materials" - Project completed.

10. DR 6 - Col. E. M. Wansbrough

"To study the effects of altitude acceleration and deceleration on oral tissues"

COL. WANSBROUGH presented a final report on this project which  
appears in APPENDIX "A"







THE CHAIRMAN inquired whether Col. Wansbrough intended to publish the results of this study and was informed that no thought had been given to this subject. Dr. Ellis said that the Committee was very happy to have had this project carried forward by the Royal Canadian Dental Corps without expense to the Committee and he thought that everyone was well pleased with the results obtained.

11. DR 7 - Dr. J. S. Bagnall

"Investigation and critical report on dental caries research" - Project completed.

THE SECRETARY presented members with copies of the booklet Trends in Caries Research and showed them sample pages from the Bibliography on Caries Research both of which reports had been prepared by Dr. Bagnall.

12. DR 8 - Dr. A.D.A. Mason

"D.M.F. caries incidence in Brantford children" - Project completed.

13. DR 9 - Drs. O. J. Walker and H. R. MacLean

"An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry".

DR. MacLEAN presented a summary indicating that the final report on this project had been submitted to the Committee at its October 1949 meeting and that a paper had been accepted by the Journal of the Canadian Dental Association reviewing this subject. The summary report appears in APPENDIX "C"

14. DR 10 - Dr. G. M. MacDonald

"Facial prostheses of vinyl resins" - Project completed.

15. DR 11 - Dr. R. J. Godfrey

"An anatomical, histological and physiological study of the role of the condyle in mastication" - Project completed.

16. DR 12 - Dr. A.E.R. Westman

"Cements for methacrylate inlays"

THE CHAIRMAN presented a summary on this project submitted by Dr. A.E.R. Westman reporting that this project is being held in abeyance pending certain experimental clarification of the properties of acrylic fillings. This summary appears in APPENDIX "H"







17. DR 13 - Dr. M. Doreen Smith

"A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario"

DR. SMITH presented a summary and a complete report indicating that the project is complete at this stage. The summary and the report appear in

APPENDIX "N"

DR. RAE inquired regarding the particle size of the ground material and was informed that all was required to pass through a 60-mesh screen, this fineness having been indicated in the literature.

DR. BROWN said that the second survey in Brantford and Sarnia would be completed by March 1951. He would like to have extracted teeth from these areas to determine whether there is an increase in fluorine content and that he would like to continue examinations of this kind for about ten years.

DR. WALKER suggested that fraternal twins (i) who had been fed pabulum and (ii) who had not been fed pabulum might be studied. She thought that some of the cases being investigated under her direction might be made available for this purpose.

DR. MacLEAN said that in his work he had found a great variation in the fluorine content of potable waters in western Canada and he wondered whether Dr. Smith had made analyses of the water supplies used by her patients.

DR. SMITH said that all water analyses had been checked with the Ontario Department of Health. She said that when new chemicals and new glassware were used it was necessary to run several blanks as there were many ways in which traces of fluorine could be lost.

DR. NUTLAY referred to para. 4, page "N-3" and suggested that this item regarding high fluorine content and mottling of a tooth should be amplified as it was very important.

DR. RAE complimented Dr. Smith on having succeeded in developing a satisfactory method for the determination of fluorine.

18. DR 14 - Dr. M. Doreen Smith

"Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries" - Project completed.

19. DR 15 - Dr. Norma Ford Walker

"A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches"







DR. WALKER presented a summary of this work which appears in  
APPENDIX "Q"  
She illustrated her talk with a number of slides.

20. DR 16 - Dr. C. H. Best

"In vivo studies of certain fungus-like micro-organisms found in periodontal disease" - Project completed.

21. DR 17 - Dr. R. F. Shaner

"An investigation of the effects of materials used in operative dentistry on the vital pulps of teeth" - Project discontinued pending the finding of a successor to Dr. Shaner to carry on the work.

22. DR 18 - Dr. H. K. Box

25. DR "Experimental artificial production of dental caries in vitro"

DR. HUGILL presented a summary and complete report on this subject which appear in APPENDIX "S"

He also outlined proposed experimental work for the term 1950-51, providing a renewal of this grant was approved. The outline of experimental work is included as a supplement to appendix "S", for purposes of record.

DR. HAM said that this project had begun as a morphological study but that it had gradually been extended until now it appeared to be merging into biochemistry where a great deal of experience would be needed if any progress were to be made.

DR. RAE drew attention to item 3 on page S-8 and said that he had been unable to prepare aqueous solution of several of the acids mentioned. At acid ranges of 4.5 pH these materials do not dissolve. He also questioned item 1 of the conclusions on page S-9.

THE CHAIRMAN thanked Dr. Hugill for his presentation.

23. DR 19 - Dr. J.K.W. Ferguson

"Local anaesthetics for small nerve-block"

THE CHAIRMAN stated that Dr. Ferguson had expected to be present at this meeting but unfortunately had been unable to come. In his absence the Chairman read the summary and the final report on this project which appear in APPENDIX "B"







24. DR 20 - Dr. Jules Thébaud

"Improvement of sub-gingival curettage performed in the treatment of the periodontal pockets"

THE CHAIRMAN presented a summary and a complete report submitted by Dr. Thébaud which appear in APPENDIX "L"

He also reported that Dr. Thébaud had recently written resigning his grant and regretting that he was unable to continue the investigation because of his impaired health. The Chairman stated that the Patents Branch of the National Research Council had investigated the possibility of securing patents on various instruments developed by Dr. Thébaud but that it had been found undesirable to proceed with patent claims on behalf of the Council. Dr. Thébaud had been informed of this decision and had been advised that if he himself wished to proceed towards securing patents on his instruments, he was quite free to do so.

25. DR 21 - Dr. A.E.R. Westman

"Investigation of repair techniques for methacrylate dentures"

THE CHAIRMAN presented the summary and more complete report which appear in APPENDIX "I"

He noted that some further studies remain to be done on this subject but that they will be completed by 31 March, 1950 at which time a final report will be submitted to the Committee, and the project discontinued.

26. DR 22 - Dr. C. H. Best

"An investigation of the mechanism of repair of the supporting structures of the teeth"

THE CHAIRMAN presented the summary by Dr. Best and the report by Dr. Linghorne and Mr. O'Connell which appear in APPENDIX "F"

DR. HAM noted that longitudinal sections had been used but he was of the opinion that cross-sections would have been preferable. He said that Dr. Linghorne had worked very hard and the report showed interesting results.

THE CHAIRMAN observed that the work had been done on animals whose tissues previous to the formation of the pocket were healthy, that is, a surgical pocket was produced by the removal of bone. He questioned whether this procedure produced the same situation as would be created by the deterioration of tissue due to disease.







DR. WATT said that Dr. Linghorne's work showed reattachment between the cementum and bone.

DR. NUTLAY questioned whether this was bone or secondary cementum.

THE CHAIRMAN said that Dr. Linghorne had shown his report to Schour and that subsequently Dr. Best had received a very complimentary letter regarding this project.

27. DR 23 - Dr. C. P. Leblond

"Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon"

THE CHAIRMAN read the summary report prepared by Dr. Leblond which appears in APPENDIX "D"

28. DR 24 - Dr. C.H.M. Williams

"The effects of hard and soft diets on the supporting structures of the teeth"

DR. D.G. WATT presented the summary and complete report on this project which appear in APPENDIX "K"

THE CHAIRMAN thanked Dr. Watt for having come to Ottawa to present this report.

29. DR 25 - Dr. A.E.R. Westman

"Direct acrylic fillings"

DR. O.W. ELLIS read the summary report on this project which appears in APPENDIX "J"

30. DR 26 - Dr. R.E. Moyers

"Measurement of physiological forces in the mouth"

THE CHAIRMAN presented the summary and read the report by Dr. Moyers which appear in APPENDIX "O"

He expressed the hope that it might be possible to invite Dr. Moyers to attend the next meeting of the Committee and to report in person on this project.

31. DR 27 - Drs. J.W. Abbiss and A.G. Nutlay

"A histological study of tooth germ development, both intra- and extra-alveolar with a survey of the pathological changes in the developing tooth"







DR. NUTLAY presented a brief summary of the work on this project and then spoke at length in explanation of the work which he and the grantee, Dr. Abbiss, have been doing at the Pathological Institute in Halifax. He expressed his warm greetings to the Committee and said how much Dr. Abbiss and he appreciated the support which had been given to their work by the Associate Committee on Dental Research. The summary report and a copy of Dr. Nutlay's presentation statement appear in APPENDIX "E"

DR. HAM said that Dr. Nutlay had brought up many items that demanded further study.

THE CHAIRMAN said he feared the field was so broad it might be too wide in scope to be definitive.

DR. NUTLAY replied that the project could be limited to two main parts if that appeared to be desirable.

32. DR 28 - Dr. O. J. Walker

"Development of a photoelectric colorimetric method for determining small amounts of fluorides"

DR. MacLEAN presented a summary and report which appear in APPENDIX "P"

33. DR 29 - Dr. M. Doreen Smith

"Biological absorption of fluorine"

DR. SMITH presented a summary and read her complete report which appear in APPENDIX "M"

DR. CLEGG suggested that amberlite might be tried as an absorbent fluorine.

DR. RAE remarked that the report represented a great deal of work. He noted that the content of fluorine in one brand of baking powder was reported at 25 p.p.m. and said he thought this was in excess of the limits permitted under the Food and Drugs Act regulations. He noted too that both pablum and pabena fluorine contents would result in more than the legal limits in the final food products.

DR. SMITH thought these high fluorine contents might be serious in areas where fluorine is present in the water and soil and she wondered whether any information was available regarding the possible interference of pablum with fat and protein metabolism in infants up to six weeks although she said the fluorine content might be useful when teeth formation was in progress provided there was no fluorine in the water supply used.







34. DR 30 - Dr. Norma Ford Walker

"The study of the closure of space following premature loss of deciduous teeth"

DR. WALKER presented the summary of her report which appears in APPENDIX "R"

She said that Dr. Liu had done excellent work on this project and she expressed the hope that Dr. Liu might be invited to present the report at the next meeting.

THE CHAIRMAN said it had been suggested that cephalographic studies should be carried on concurrently with this project to determine not only the movement of the teeth but also the formation of the arch. To this end it would be necessary to secure the co-operation of the radiology and orthodontic departments.

35. Publications (Min. 37, 10th Mtg.)

THE CHAIRMAN reported that a preliminary list of the publications of the Committee had been distributed in December 1949 to the members and that a revised list would be issued in each subsequent set of proceedings. The present list appears in APPENDIX "U"

THE CHAIRMAN stated also that in accordance with the recommendation of the Executive (Min. 6, 7th Exec.) a summary of the proceedings of the 10th Meeting had been prepared by the Secretary and published in the Journal of the Canadian Dental Association through the courtesy of Dr. Garvin. A similar statement would be prepared for publication regarding this and subsequent meetings of the Committee.

36. Finances

THE CHAIRMAN reported on the financial standing of the Committee as of 31 January 1950 as follows:

|                          |             |
|--------------------------|-------------|
| Funds available, 1949-50 | \$35,500.00 |
| Expenditures             | 22,154.99   |
| Commitments              | 11,331.95   |
| Free balance             | 2,013.06    |

He presented a summary of awards and expenditures to date for the information of the Committee. This statement appears in APPENDIX "T"







He also reviewed the individual grants and noted that in a few instances the grantees had not taken up the full amount of their awards which meant that the Committee had some additional funds in reserve from these sources. He suggested that as the applications in hand for 1950-51 amounted to a total greater than the Committee would have available for grants in that year, and as some of the new applications contained items of equipment that should be readily available, it might be possible to purchase some of these items of equipment out of 1949-50 funds. In order to do this it would be necessary to make supplementary grants and to ask the grantees to arrange for the purchase of special items of equipment which they would need in 1950-51 before 31 March 1950 which is the end of the Government's fiscal year.

DR. THOMSON thought this was an excellent suggestion but that it should be restricted to those grantees who were applying for renewals of awards. The Committee AGREED with these suggestions.

The First Session adjourned at 5 P.M., and the Chairman announced that the Executive would meet at the Chateau Laurier at 7:30 P.M., that evening to consider applications.

SECOND SESSION  
3 March, 1950

37. Attendance

Dr. R. G. Ellis, Chairman

Dr. H. K. Brown

Dr. E. Charron

Dr. J. B. Collip

Dr. O. W. Ellis

Dr. M. H. Garvin

Dr. A. W. Ham

Dr. H. R. MacLean

Dr. R. H. McDougall

Dr. J. J. Rae

Dr. D. L. Thomson

Col. E. M. Wansbrough

Mr. S. J. Cook, Secretary

By Invitation

Dr. A. G. Nutlay

38. Chairman's Remarks

THE CHAIRMAN welcomed Dr. Collip. He said that a meeting of the Executive had been held on the preceding evening and the recommendations of the Executive regarding applications for grants would be presented at this meeting.







39. Applications for Grants-in-aid

(a) RENEWALS

The following applications for renewals of grants were approved (or otherwise dealt with) as indicated hereunder:

| <u>Grant No.</u> | <u>Grantee</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <u>Amount (1950-51)</u><br>\$ |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| DR 1             | Dr. J. J. Rae,<br>Dr. Rae's application was for \$1500 but the Committee decided to vote him \$550 out of 1949-50 funds.                                                                                                                                                                                                                                                                                                                                                                                               | 950                           |
| DR 2             | Dr. G.H.W. Lucas                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 1550. <sup>2</sup>            |
| DR 12)           | Dr. A.E.R. Westman                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                               |
| 25)              | (Application for \$4,000. The Executive recommended a reduction of the grant to \$3,000 with a suggestion that the Ontario Research Foundation be asked to allocate this amount between the two grants as they see fit. It was felt the project was approaching completion in so far as the Committee's interest were concerned.)                                                                                                                                                                                      |                               |
| DR 13            | Dr. M. Doreen Smith                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1475.                         |
| DR 15            | Dr. Norma Ford Walker                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 800.                          |
| DR 18            | Dr. H. K. Box<br>(The Executive recommended that this application for \$2120 should not be granted. There was considerable discussion on this application. It was pointed out that the research has been going on for some time and that the morphological side appears to have been completed and to have been done well. Biochemical work proposed is a new phase that may be outside the field of specialization of the grantee. The mechanism for in vitro production of dental caries seems to have been proved.) | --                            |

It was MOVED by Dr. Thomson and SECONDED by Dr. Charron,

That the application in its present form be not accepted but that Dr. Box be informed the Committee would be prepared at a later date to consider a revised application and projected programme

and

That the Executive be authorized to deal with any such application.

CARRIED.







| <u>Grant No. (cont'd)</u> | <u>Grantee</u>                                                                                                                                                                                                                                                                                                | <u>Amount, 1950-51</u><br>\$ |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| DR 22                     | Dr. C. H. Best<br>A renewal (\$2600) and a new application (\$ 1500) were received. Executive recommended these applications be combined, that \$130 be granted as a supplemental vote from 1949-50 funds and that the new application be reduced from \$1500 to \$1200 making a total of \$3800 for 1950-51. | 3800.                        |
|                           | It was MOVED by Dr. Thomson and SECONDED by Col. Wansbrough,                                                                                                                                                                                                                                                  |                              |
|                           | That \$3930 (including \$130 from 1949-50 funds) be granted under DR 22 with advice to the applicant that the Committee expects him to proceed with the fusospirochetal studies as well.                                                                                                                      | CARRIED.                     |
| DR 23                     | Dr. C. P. Leblond<br>Application for \$2800. It was AGREED to grant \$400 out of 1949-50 funds and balance from 1950-51.                                                                                                                                                                                      | 2400.                        |
| DR 24                     | Dr. C. H. M. Williams                                                                                                                                                                                                                                                                                         | 1735.                        |
| DR 25                     | Dr. A. E. R. Westman (See DR 12)                                                                                                                                                                                                                                                                              |                              |
| DR 26                     | Dr. R. E. Moyers<br>It was MOVED by Dr. Ham and SECONDED by Dr. MacLean,                                                                                                                                                                                                                                      | 150.                         |
|                           | That an additional grant of \$150 from 1949-50 funds approved by the Executive be CONFIRMED.                                                                                                                                                                                                                  |                              |
| DR 27                     | Dr. J.W. Abbiss and Dr. A.G. Nutlay<br>Application was for \$300 but on discussion this was reduced to \$200.                                                                                                                                                                                                 | 200.                         |
| DR 28                     | Dr. O. J. Walker                                                                                                                                                                                                                                                                                              | 1000.                        |
| DR 29                     | Dr. M. Doreen Smith<br>On the recommendation of the Executive this application for \$1500 was <u>NOT</u> approved.                                                                                                                                                                                            |                              |







| Grant No. (cont'd) | Grantee               | Amount (1950-51) |
|--------------------|-----------------------|------------------|
| DR 30              | Dr. Norma Ford Walker | \$1400           |

On the recommendation of the Executive it was agreed to reduce this application of \$2600 to \$1400 which includes \$1200 for salaries and \$200 for supplies.

Dr. Garvin asked whether any work was being done on irregularities arising from use of space retainers (when molars are lost prematurely).

Dr. Thomson thought the point was well taken but that application for a grant for such work should come from a dentist.

#### (b) NEW APPLICATIONS

|       |                                       |      |
|-------|---------------------------------------|------|
| DR 31 | Mr. G. J. Miller and Dr. L. B. Jaques | 1870 |
|-------|---------------------------------------|------|

"An investigation of nerve block"

|       |               |     |
|-------|---------------|-----|
| DR 32 | Dr. A. W. Ham | 900 |
|-------|---------------|-----|

"Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals"

Application was for \$1720 but on the recommendation of the Executive it was AGREED to reduce this grant to \$900.

|       |                  |      |
|-------|------------------|------|
| DR 33 | Dr. H. A. Hunter | 1500 |
|-------|------------------|------|

"A study of the calcifying potential of calcium oleate on the dental pulp"

On the recommendation of the Executive this application for \$2500 was reduced by cutting the amounts for expenses and for the technician in half.

|       |                |      |
|-------|----------------|------|
| DR 34 | Dr. A. D'Iorio | 2840 |
|-------|----------------|------|

"Studies of calcium metabolism in tooth with the aid of Ca<sup>45</sup>"

On the recommendation of the Executive this application for \$3340 was reduced by a general cut of \$500.







| Grant No. (cont'd) | Grantee                                                                                           | Amount (1950-51)<br>\$ |
|--------------------|---------------------------------------------------------------------------------------------------|------------------------|
| DR 35              | Dr. J. B. MacDonald<br>"Experimental fusospirochetal infection<br>with mixtures of pure cultures" | 2000.                  |

On the recommendation of the Executive this application was reduced from \$3150 to \$2000.

Dr. Ham thought the allowance for dry ice was very high and suggested that it would be more economical over a period of years to purchase a deep freeze for the preservation of cultures.

#### 40. Amounts of Grants 1950-51

Total amount voted

(a) Renewals \$18,460.

(b) Applications 9,110.  
TOTAL 27,570.

In addition three supplementary grants totalling \$1080. were made from 1949-50 funds for the purchase of equipment required in 1950-51.

#### 41. Fellowships (Min. 5, 7 Exec.)

THE CHAIRMAN reported that in accordance with the decision of the Executive, application forms for grants and fellowships were mailed to the dental schools and previous grantees. It was a disappointment to have to report that no applications have been received for the fellowships offered by the Committee despite the fact that the subject had been talked about in all dental schools in the hope of interesting some eligible candidates to apply for fellowships. It appeared that the emolument offered under the fellowships scheme was too low to attract men away from private practice and other dental appointments.

#### 42. Membership

##### (i) Resignation of Dr. Stewart

THE CHAIRMAN presented a letter of resignation from Dr. C. B. Stewart, Director of Health Survey, Department of Public Health, Halifax, N.S. Dr. Stewart stated that he now found it impossible to attend meetings of the Committee because of the pressure of other duties and he felt it was desirable that he should be replaced on the Committee as he did not wish to retain a purely nominal membership. Dr. Stewart's term of membership extends to 31 March, 1951.







It was MOVED by Dr. Collip and SECONDED by Dr. Thomson,

That the Committee accept with regret Dr. Stewart's resignation. CARRIED.

After a brief discussion as to a suitable nominee in place of Dr. Stewart

It was MOVED by Dr. Garvin and SECONDED by Dr. Charron,

That the Executive be empowered to nominate a successor for the remainder of Dr. Stewart's term. CARRIED.

(ii) Changes in Membership, 1950

THE CHAIRMAN also reported that the terms of five members of the Committee expire 31 March, 1950. The members affected are Dr. Best, Dr. Garvin, Dr. MacLean, Dr. Rae and Dr. Thomson. The Executive found that each of these members is eligible for re-appointment and recommended that all five be nominated for a further term of three years on the Committee membership.

It was MOVED by Dr. Charron and SECONDED by Dr. Ellis,

That the Committee concur in the recommendation of the Executive that Dr. Best, Dr. Garvin, Dr. MacLean, Dr. Rae and Dr. Thomson be invited to accept nomination for a second term and on their acceptance they be renominated as members of the Associate Committee on Dental Research for the three years ending 31 March, 1953. CARRIED.

It was MOVED by Dr. Thomson and SECONDED by Dr. McDougall,

That in the event of any of the retiring members declining renomination, the Executive be empowered to make new nominations. CARRIED.

43. Air Travel (Min. 41, 10th Mtg.)

THE CHAIRMAN reported that Dr. Nutlay, who had been invited to attend this meeting, had travelled by air and in order that his expenses might be paid it was necessary to have the approval of the Committee for air travel.

It was MOVED by Dr. Ellis and SECONDED by Dr. MacLean,

That air travel be authorized for Dr. Nutlay's attendance at the 11th Meeting of the Committee. CARRIED.

It was MOVED by Col. Wansbrough and SECONDED by Dr. Thomson,

That travel by air be authorized for 1950-51 in the case of Dr. Garvin, Dr. MacLean and Dr. McDougall

AND







That the Executive be empowered to deal with any further cases of air travel as they may arise during 1950-51. CARRIED.

#### 44. New Business

##### (i) Ammoniated Dentifrices

DR. GARVIN inquired regarding the publication of Dr. Rae's work especially that part which related to the use of ammoniated dentifrices.

DR. BROWN said that the findings of the American Dental Association on the use of ammoniated dentifrices had been accepted by the Canadian Dental Association.

DR. RAE referring to an item of his report (Appendix "G" page 4) said he was not yet ready to publish his results.

##### (ii) New Research Projects

DR. GARVIN mentioned that the selection of new projects for work under the Committee is based on applications received but he wondered if it would not be within the province of the Committee to suggest programmes of research which might be taken up by applicants for grants. He mentioned for example that considerable interest exists in the problems involved when gold inlays are made over amalgams. There is little information as to whether this causes disintegration of the cement.

THE CHAIRMAN said that he would put an item of new research problems on the agenda for the next meeting of the Executive.

#### 45. Date of Next Meeting

It was MOVED by Dr. Garvin and SECONDED by Dr. MacLean,

That Monday and Tuesday, 16-17 October, 1950, be tentatively adopted as the dates of the next meeting of the Committee. CARRIED.

The Meeting adjourned at 12:40 P.M.







## APPENDIX "A"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

January, 1950

Subject: To study the effects of decreased barometric pressure on the oral tissues.

Authors: R.C.D.C.

Submitted  
by: E. C. Purdy, Major

Project: DR 6

#### INTRODUCTION

Dental problems associated with flying were recognized and reported by many observers in the period prior to World War II.

The first published report (1937) (1) associating toothache with altitude, described the case of a pilot who experienced toothache each time he ascended above 1800 meters. A pulpitis in a lower molar was observed and a pulpectomy performed. The pilot was then able to resume his flying without further symptoms. The pain was ascribed to a simulated suction acting upon the inflamed pulp through lowered barometric pressure.

In 1939 (2.3) it was observed that normal teeth were unresponsive to changes in altitude but teeth displaying pain showed some pulpal or periodontal pathology. The pain was attributed to a lower pressure and it was suggested that the patient should blow up his cheeks to get relief.

The increased interest in aviation problems and the frequency of dental complaints among flying personnel during the training of large numbers of men in the recent war was sufficient to elicit numerous reports as to its frequency and cause.

Brickman (4) studied 2300 subjects in the low pressure chamber and reported twenty-seven cases of aerodontalgia or 1.17 per cent. The cause in most cases was described as due to recurrent caries under old fillings involving or nearly involving the pulp.

A study of 3,428 US Marine Corps personnel during routine indoctrination in the low pressure chamber (5) revealed the incidence of aerodontalgia to be 1.86%. It was concluded that the majority of cases of dental pain was the result of referred pain from the maxillary sinus.



The U.S.A.A.F. Dental Research Group (6) published a comprehensive report in 1946 of the work of numerous investigators working independently or in organized groups. They concluded that high altitude environment does not affect a normal pulp. Aerodontalgia requires a pre-existing pathologic disturbance of the pulp except for referred pain from aero-sinusitis or aero-otitis.

Various investigators have reported the incidence of dental complications during flight conditions to be from less than 1% to more than 2% of all subjects tested. This would place dental problems fifth in frequency of all complications resulting from flight up to an altitude of 30,000 ft. Bends, aero-otitis, abdominal distress and sinus pain have been reported in greater frequency. If however the incidence of dental pain is considered in relation to the entire flying experience of an individual, its occurrence assumes much smaller proportions. The infrequent occurrence of such problems would seem to limit the importance of this field of study, but the severity and persistence of the pain when it does occur demands a thorough understanding of its cause, prevention and treatment.

Pulp stones, air trapped under fillings, traumatic occlusion, vibration, lowered temperature and others have been suggested as possible causes of aerodontalgia. Many of the above have already been satisfactorily disproven.

The use of a decompression chamber for the study of aerodontalgia permits only the simulating of flying conditions in so far as it concerns atmospheric pressure. Vibration, nervous tension, rapid acceleration and deceleration which may accompany actual flight cannot be considered in this study.



"A-3"

Typical sheet of recorded data of four RCAF flight cadets subjected to tests in the decompression chamber.

DATE 17 Sept. 47

Intake 2nd.

|                         |             |                |               |              |
|-------------------------|-------------|----------------|---------------|--------------|
| NAME .....              | MARK J S .. | SZIEZAK. MS... | HOUSTON.WD... | GUERIN JG... |
| NUMBER.....             | 16500       | R-253450       | U-278461      | 1594         |
| AGE .....               | 18          | 22             | 21            | 19           |
| FLYING TIME .....       | 6 hrs.      | 140 hrs.       | 120 hrs.      | 5 hrs        |
| HYGIENE .....           | FAIR.       | FAIR.          | FAIR.         | FAIR         |
| NO. MISSING TEETH.....  | NIL         | 4              | 2             | 2            |
| NO. REPLACED TEETH..... | NIL         | NIL            | NIL           | NIL          |
| CALCULUS .....          | NIL         | SLIGHT         | MODERATE      | ABUNDANT     |

|                        |          |        |              |        |
|------------------------|----------|--------|--------------|--------|
| PARTIALLY ERUPTED      |          |        |              |        |
| OR IMPACTED TEETH..... | NIL      | NIL    | /8           | NIL    |
| LARGE.....             | /6 (do)  | NIL    | 6/ (mo)      | NIL    |
| MEDIUM .....           | 4        | 8      | 3            | NIL    |
| SMALL .....            | 13       | 13     | 2            | 5      |
| LAST FILLING INSERTED. | 1 yr.    | 2 yrs. | 4 yrs.       | 2 yrs. |
| OCCCLUSION             | ANGLE 2  | NORMAL | TRAUMATIC    | NORMAL |
|                        | SLIGHT   |        | ANTERIOR END |        |
|                        | OVERBITE |        | TO END BITE  |        |

|          |            |        |            |           |
|----------|------------|--------|------------|-----------|
| FUNCTION | NORMAL     | NORMAL | NORMAL     | NORMAL    |
| GINGIVAE | MILD       | NORMAL | IRRITATED  | IRRITATED |
|          | GINGIVITIS |        | CHRONIC    |           |
|          |            |        | GINGIVITIS |           |

|                   |        |        |             |               |
|-------------------|--------|--------|-------------|---------------|
| BLEEDING TENDENCY | SLIGHT | NIL    | MODERATE    | SLIGHT        |
| LESION            | NIL    | NIL    | SIMPLEX     | NIL           |
|                   |        |        | PERIODONTIS |               |
| SUPPORTING BONE   | NORMAL | NORMAL | NORMAL      | NORMAL        |
| TOOTH MOBILITY    | NORMAL | NORMAL | NORMAL      | NORMAL        |
| EXPOSURES OR      | NIL    | NIL    | NIL         | 6/ (mod) SEN- |
| NEAR EXPOSURES    |        |        |             | SITIVITY LESS |
|                   |        |        |             | THAN NORMAL   |
| LARGE             | NIL    | NIL    | NIL         | NIL           |
| MEDIUM            | NIL    | NIL    | NIL         | 4             |
| SMALL             | 3      | 4      | 5           | 5             |

|                        |         |     |     |     |
|------------------------|---------|-----|-----|-----|
| PULPITIS               | NIL     | NIL | NIL | NIL |
| X-RAY                  | NIL     | NIL | NIL | NIL |
| NON - VITAL TEETH      | NIL     | NIL | NIL | NIL |
| 1ST TEST to 30,000 ft. | GENERAL | NIL | NIL | NIL |
|                        | DENTAL  | NIL | NIL | NIL |

|                  |     |     |     |              |
|------------------|-----|-----|-----|--------------|
| DENTAL TREATMENT |     |     |     |              |
| BETWEEN TESTS    | NIL | NIL | NIL | 6/ (mod) Ce. |
| GENERAL          | NIL | NIL | NIL | NIL          |
| DENTAL           | NIL | NIL | NIL | NIL          |

|                        |  |  |  |  |
|------------------------|--|--|--|--|
| 2nd TEST TO 30,000 ft. |  |  |  |  |
|------------------------|--|--|--|--|



- 1 Three hundred and fifty-three flight cadets of the R.C.A.F. were dentally examined and oral conditions were recorded (see accompanying chart).
- 2 They were subjected to runs in the decompression chamber during routine air crew tests.
- 3 All subjects displaying dental symptoms during decompression were interviewed and re-examined.

At no time was the possibility of toothache suggested to any of the subjects prior to their run in the decompression chamber, persons suffering from head colds, sinusitis or other contra indications were eliminated from the test until a future date. The age group of subjects varied from 18 to 26 years.

Prior to the decompression test, an explanatory lecture was given to familiarize the subjects with the chamber and the use of the oxygen equipment.

A routine decompression test begins with a simulated ascent to 5,000 ft. then lowered to ground level to eliminate subjects unable to equalize the pressure in the middle ear. If all is well, the pressure inside the chamber is then lowered at a steady rate simulating a climb of 2,000 ft. per minute until an altitude of 17,000 ft. is reached. It is held at this level for about five minutes and oxygen masks are placed and adjusted. The climb then continues at the same rate or less until an altitude of 30,000 ft. or more is reached. After a brief interval of five minutes or less a steady rate of descent takes place at 2,000 ft. per minute until ground level is reached.

As the occasion demands, variations in the run are made to relieve painful symptoms in ears, sinuses, teeth, etc. This usually involves a levelling off, descent or re-ascent to approximately the altitude where the painful symptoms occurred.

All subjects exhibiting dental pains were immediately re-examined clinically and radiographically and details of the symptoms recorded. They were also questioned regarding previous dental pains, sinus pains, recent fillings, presence of mild colds etc.

If referred pain from the maxillary sinus was suspected, the subject was instructed in the use of the Valsalva procedure. This consists of creating a positive pressure in the oral and nasal cavities by attempting to expel air with the mouth closed and the nostrils pinched. The subject was given a second run in the chamber at a later date and no dental work was performed in the interval.

#### RECORDED DATA

The total number of subjects examined dentally prior to decompression tests in this survey totals 353. The dental condition of this group is illustrated in the accompanying table.



"A-5"

# DENTAL CONDITIONS OF 353 R.C.A.F. PERSONNEL

## SUBJECTED TO DECOMPRESSION TESTS

NUMBER OF MISSING TEETH EXCLUSIVE OF THIRD MOLARS ..... 364

NUMBER OF PARTIALLY ERUPTED THIRD MOLARS ..... 91

NUMBER OF CAVITIES VERY LARGE ..... 123  
MEDIUM ..... 501  
SMALL ..... 1196

TOTAL NUMBER OF CAVITIES 1820 5.15 per mouth

NUMBER OF FILLINGS VERY LARGE ..... 335  
MEDIUM ..... 702  
SMALL ..... 2198

TOTAL NUMBER OF FILLINGS 3235 9.15 per mouth

NUMBER OF TEETH WITH PULP EXPOSURE  
NEAR EXPOSURE OR IRREPARABLE 86

NUMBER OF MOUTHS WITH MARKED TRAUMATIC OCCLUSION ..... 123

NUMBER OF KNOWN NON-VITAL TEETH OR ABSCESSSED TEETH .... 12

NUMBER OF SUBJECTS WITH RECENT FILLINGS INSERTED ..... 19  
(Less than three weeks.)

NUMBER OF SUBJECTS WITH NO RESTORATIVE DENTISTRY  
BUT IN GROSS NEED OF IT ..... 21

NUMBER OF MOUTHS HYGIENICALLY NEGLECTED ..... 75

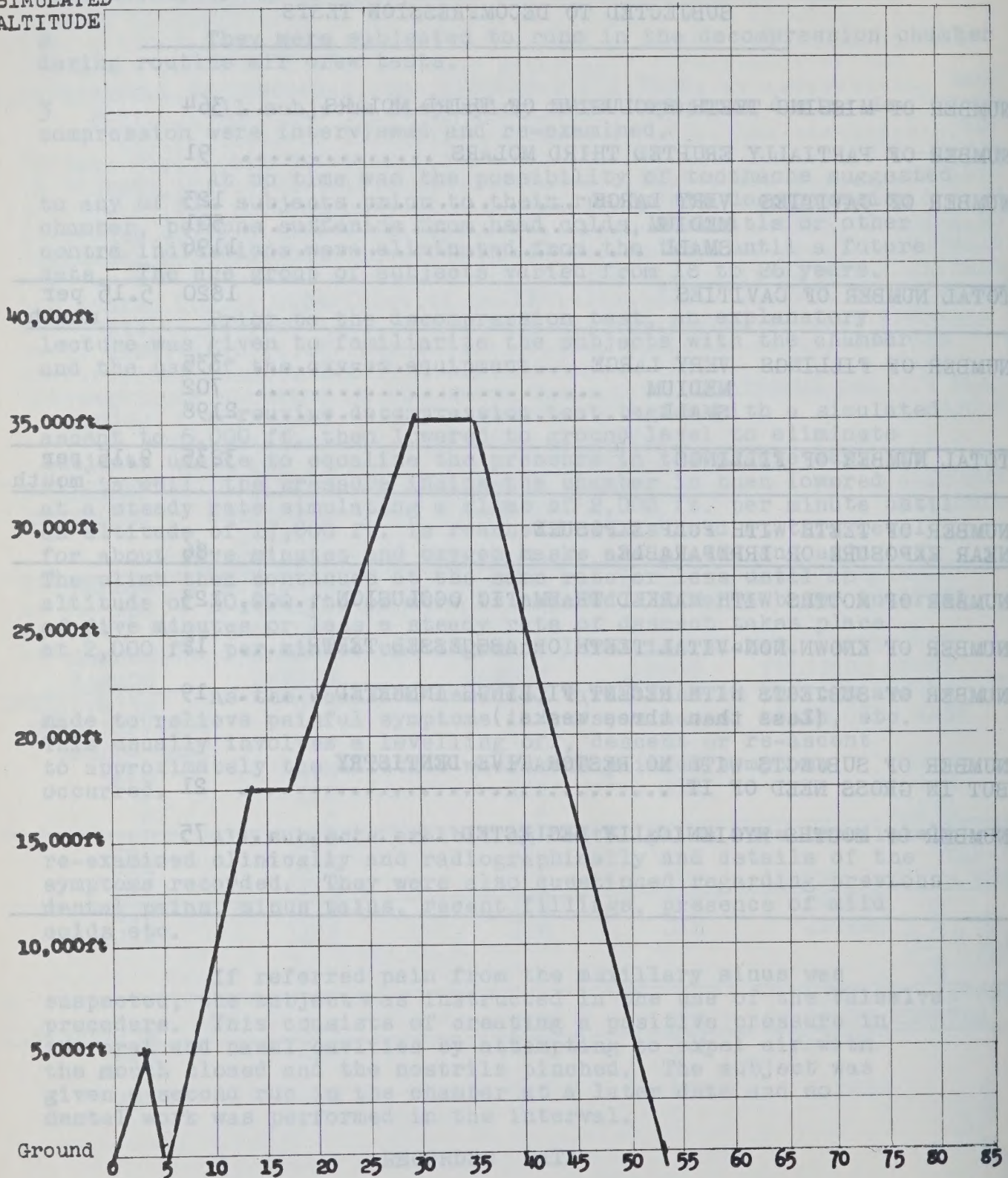
This was the individual who did not have symptoms of a cold after the previous run but described a history of sinusitis.



"A-6"

# A GRAPHIC ILLUSTRATION OF A ROUTINE RUN IN THE DECOMPRESSION CHAMBER

SIMULATED  
ALTITUDE





The majority of the group were only recently inducted into the R.C.A.F. prior to being examined, which accounts for the great need for dental attention. Of this group many presented a picture of gross dental neglect and 21 or 6% had experienced no previous dental treatment other than extraction of carious or painful teeth.

The total number of subjects in this group describing symptoms related to the teeth as a result of the decompression test were 8 or 2.8%. Of these 8, only 6 described pain in the teeth or 2.1%. The other two cases are described below.

Case 1. Pain with pronounced swelling in the right temporal region at a simulated altitude of 25,000 ft. and above occurred two days after the surgical removal of a lower right impacted third molar. Swelling subsided at normal atmospheric pressure but tenderness and crepitation remained for 48 hours. This subject was a member of the staff taking frequent runs in the chamber with the indoctrinated groups and is included in this report as a matter of interest. Patient was grounded for two weeks and presented no symptoms on later runs in the chamber.

Case 2. One subject described pain and tenderness in the upper right second bicuspid area twelve hours after a decompression test. Examination of the area revealed a non-vital tooth with early apical abscess. Extraction of this tooth alleviated the symptoms. No pain had been experienced during the decompression test.

Of the six subjects describing symptoms of aerodontalgia while in the chamber, two described the pain as occurring while ascending and four described the pain as occurring while descending.

All four experiencing pain while descending described the area as the upper bicuspid or first molar region. All described the onset of pain between 18,000 and 25,000 feet. All described gradual relief after reaching ground level but a dull ache or tenderness continued for several minutes after leaving the chamber. Three of the four described having or just recovering from a mild head cold. The other described previous attacks of maxillary and frontal sinusitis but displayed no symptoms of a cold. Two of the four had no cavities or dental pathology in the area where the pain seemed localized and no deep fillings were present. The other two displayed only moderately deep cavities, one very large filling in an upper first molar but no pathological lesions could be found. All four were subjected to a second run in the chamber after an interval of 7 to 14 days and three displayed no symptoms of pain. The fourth had a similar experience to his first test but found relief from the Valsalva procedure. This was the individual who did not have symptoms of a cold after the previous run but described a history of sinusitis.



The two subjects describing toothache while ascending described the area as the lower third molars. Both described symptoms occurring after reaching 20,000 ft. or more and continuing with the increased altitude. In both cases the pain was relieved or disappeared on returning to or slightly below the level at which the pain first occurred. In both instances the lower third molars had been very recently filled with amalgam and no cavity lining was inserted. Although only moderately deep the teeth were described as being very sensitive during cavity preparation and a local anaesthetic was required in the one instance. The teeth were also very sensitive to cold and no other dental defects or very deep restorations were present in the area. Three weeks later, the sensitivity to cold had disappeared and a second run in the chamber displayed no symptoms of pain.

#### DISCUSSION OF DATA

The incidence of dental pain occurring in the low pressure chamber for this group was six out of 353 or 1.7%. Because of the comparatively small number on which this study was based, the incidence factor cannot be considered as a representative figure although it is quite in accordance with reports from other observers.

Of 86 teeth irreparably damaged by caries and having the pulp exposed or involved in the process, not one displayed any signs of discomfort during decompression. It is possible however that in such mouths some symptoms were experienced but the subject failed to report it for personal reasons or fear of consequences. The mouths of subjects reporting pain did not suggest a picture of gross dental neglect.

The onset of pain was usually described as quite sudden and severe and in most cases was located in the upper posterior quadrant of the mouth.

An analysis of the recorded data would seem to indicate that the pain experienced by the four subjects while descending in the chamber was due to maxillary sinus blockage. In all cases there was some confusion or uncertainty on the part of the patient as to the exact source of the pain, but all described the upper bicuspid or first molar area. Although the group was roughly screened to eliminate persons with upper respiratory infection it seems likely that greater care in screening may have reduced the incidence of this type of pain. This result conforms however to results obtained by Hutchins et al (5) where a study of 37 cases of aerodontalgia arising in the decompression chamber brought forth the conclusion that the majority of cases of dental pain under lowered pressure were caused by referred pain from the maxillary sinus. In addition Bunch et al (7) concluded that, in all probability, maxillary sinus blockage is the cause of aerodontalgia occurring on descent.



No satisfactory explanation can be offered for the cause of two cases of aerodontalgia following the recent insertion of amalgam fillings. Brickman (4) and Miller (8) attributed several cases of aerodontalgia to trauma incident to the placement of a recent filling and suggested a relationship between a hyperemic pulp and dental pain under decompression. It was also pointed out that such symptoms seemed to disappear after a few weeks.

There is no proof that the peri-apical lesion presenting symptoms of pain and tenderness, twelve hours after a run in the chamber was incited by decompression. Freitag (9) was the first to demonstrate that expanding gas in the pulp chamber or root canal of a tooth could force material through the apex. A number of cases of root canal therapy were performed and the canals filled with thorotrast leaving an air bubble sealed inside. Radiograms were taken at various altitudes and the expanding air bubble was observed to force some of the radiopaque thorotrast through the apical foramen.

A procedure similar to above was carried out by the author with a number of extracted teeth. It was found that the sealed-in-air bubble would greatly expand under decompression if permitted to do so. The forcing of material through the apex of the tooth would seem to be dependent on the following factors:

1. The size of the apical foramen or canal.
2. The ability of the canal contents to flow freely.
3. The apical foramen must be in communication with lowered barometric pressure.
4. The resistance of the tissues around the apex to displacement.

The presence of air spaces or gas bubbles in pathological pulps is a proven fact and it is conceivable that decompression could cause an acute exacerbation of an existing pathological condition. The possibility of expanding gases in the pulp chamber or root canal, forcing semi-fluid septic material through the apex is a consideration that must be kept in mind.

#### SUMMARY

1. The incidence of aerodontalgia in a group of 353 flight cadets is 1.7 %.
2. Not one of 86 teeth with pulp exposure or near exposure caused pain under decompression.
3. Four cases experiencing dental pain while descending in the chamber were attributed to blockage of the maxillary sinus.



SUMMARY (cont'd)

4. Two cases of dental pain under decompression occurred in recently filled sensitive teeth without a cavity lining.
5. The possibility of pulpal gas bubbles under lowered pressure as a factor resulting in the exacerbation of a chronic condition is discussed.

CONCLUSIONS

1. Although the incidence of dental complications during decompression is small, the possibility of such an occurrence should be kept in mind by all persons performing dental operations for personnel subjected to such conditions.
2. There is little doubt but what many individuals experiencing maxillary sinus blockage during descent interpret the pain as of dental origin.
3. The thermal, chemical or traumatic shock to the pulp accompanying cavity preparation and the insertion of a filling, may in some cases cause pain under decompression. Care in cavity preparation and the use of insulating materials in sensitive or deep cavities are suggested.
4. The expansion of gases in putrescent pulps and abscess cavities during ascent, would seem to be an activating factor in some cases of exacerbation of a chronic pathological condition or dissemination of infection.
5. No changes in the accepted methods of treatment of dental problems are suggested. A thorough and frequent dental examination accompanied by good dentistry would seem to eliminate many of the problems in the field of aviation dentistry.



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APPENDIX "B"

FINAL REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: Assessment of local anaesthetics for small nerve-block.

Grantee: Dr. J. K. W. Ferguson

Project: DR 19

SUMMARY OF WORK

Retro-ocular blocks in guinea pigs have proved to be suitable for the investigation of relative potencies of local anaesthetics. Epinephrine 1:100,000 and 1:20,000 has remarkably little effect in prolonging these blocks. The prolongation does not exceed 20%, and 1:20,000 is no better than 1:100,000. Toxicity for tissue has been tested by intradermal injection in guinea pigs. The occurrence of petechial haemorrhages seems to be a better criterion of toxicity than diameter of erythema. The various anaesthetics differ more widely in skin toxicity than in systemic toxicity, confirming our earlier conclusion that this indication of toxicity should be used in preference to systemic toxicity for evaluation of drugs for small nerve blocks. Of the drugs tested, dibucaine (nupercaine) had a significantly higher relative rating than the others.







## APPENDIX "B"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Subject: Relative ratings of local anaesthetics for small nerve blocks.

Grantees: Dr. J. F. Aikenhead and Dr. J.K.W. Ferguson  
from the Department of Pharmacology and Ophthalmology,  
University of Toronto.

Project: DR 19

Hamilton, Westfall and Ferguson (1) proposed a series of indices for the quantitative expression of the inherent safety of local anaesthetics. These were called relative rating indices. The indices were determined in different ways according to the purpose for which it was proposed to evaluate the drug. For example, for surface anaesthesia of the cornea, the systemic toxicity of the drug is seldom of importance but the concentration at which tissue damage occurs is of considerable interest. Similarly, for small regional nerve blocks, e.g. retrobulbar or mandibular blocks, systemic toxicity is less important than toxicity to tissues. Hamilton, Westfall and Ferguson used intradermal injections with increasing concentrations and measured the diameter of the erythematous reaction as an indication of tissue damage. Their observations on toxicity to skin were too scanty to allow more than tentative conclusions. One purpose of the present investigation was to extend their observations on intradermal toxicity and to evaluate their validity and accuracy. Hamilton, Westfall and Ferguson computed Relative Ratings for purposes of small nerve blocks by dividing the Relative Potency (as measured by intradermal injection) by the Relative Toxicity (for the skin). It may be that potency by intradermal injection does not give the best measure of potency for nerve block. Consequently we have attempted to get a more direct measure of potency for nerve block. Experimental measurements of this kind have been made before, e.g. by Shackell (2), using the guinea pig's sciatic nerve. In our hands this preparation gave very irregular results with many apparent failures. We have accordingly explored retro-ocular block in guinea pigs as a method of measuring potencies of local anaesthetics.

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1. Assisted by a grant from the Associate Committee on Dental Research, National Research Council, Ottawa.

2. Hermant Fellow in Ophthalmology.







## Methods

Intradermal toxicity. The various concentrations of each drug were injected in a constant volume of 0.2 cc. using a 0.25 cc. tuberculin syringe. Adult white guinea pigs were used. The skin of the back was prepared by removing the hair with fine clippers on the day before injection. From the time of clipping to the end of the experiment the animals were kept in separate cages to keep them from biting or scratching each other. Four and sometimes five concentrations of each drug were injected into the skin of one animal, usually on the same day. The series was repeated on four different animals on other days. Care was taken that the same concentrations of drug were tested at different locations on the back. For the stronger drugs, Dibucaine and Tetracaine, only two injections were given at one time to a single animal because even the small amounts used in four intradermal injections can cause systemic toxicity. The diameters of the erythematous areas were measured when at their largest size, viz 24 hours after injection. Several diameters of each erythematous area were measured and averaged. A record was kept of petechiae and ulceration at the site of injection.

Retrobulbar block. Injections were made in a constant volume of 0.2 cc., using a 26 gauge needle, 1 inch long. After a number of preliminary experiments the approach used routinely was through the skin 1/4 inch posterior and ventral to the posterior canthus of the eye. The point of the needle was inserted below the temporal ridge, behind the eyeball, and directed perpendicularly to the sagittal plane till stopped by the medial wall of the orbit. The needle was then drawn back about 1/16 inch for the injection. Observations were made on the corneal reflex, light reflex, and dilatation of the pupil. The corneal reflex was tested by means of a strip of celluloid with a smooth ball of sealing wax on the end, as suggested by Hirschfelder and Bieter (3).

Epinephrine. Some experiments were done by retrobulbar block with procaine, 1% and 2% in epinephrine 1:100,000, and also with dibucaine 0.01% and 0.02% in epinephrine 1:100,000. Each concentration was tested on five animals.

In all cases injections were made in 0.9% NaCl and in all cases the pH was between 6 and 7.

## Results

The average diameters of the erythematous areas, as recorded 24 hours after injection, are shown in Table I. The points were plotted against log concentration. Straight lines drawn through points by inspection are shown in fig. 1.







The continuous lines are replaced by broken lines, starting at the concentrations at which half the animals showed petechiae or ulceration (the T.C. 50). Since these lines are not all parallel, it is not possible to express relative toxicities by a single figure which would be applicable at all concentrations used. A decision had to be made as to what degree of reaction should be used for comparative purposes. In making this decision, consideration was given to the concentrations at which definite tissue damage appeared in the form of petechiae or ulceration. In Table I the last column shows the concentration, estimated by interpolation, at which half of the animals showed petechiae or ulceration. These figures may be used as an independent measure of toxicity and may be more relevant than the area of erythema. It is noteworthy that petechiae began to appear at a larger diameter of erythema with some drugs than with others. The diameter of erythema chosen for comparison was 6 mm., which is below the petechial reaction level for all but one drug. This degree of reaction is given by 2.3% procaine, a concentration not considered damaging in most practical applications. This question of an appropriate criterion of toxicity will be discussed later.

### Retrobulbar Block

The average durations of loss of corneal reflex are shown in Table II. It was found that pupillary dilatation and loss of light reflex lasted roughly twice as long as loss of corneal reflex. No significant differences were observed between the anaesthetics as regards time to produce anaesthesia by this method. Full corneal anaesthesia was produced usually in less than 1 minute and always in less than 3 minutes. The durations of corneal anaesthesia were plotted against log concentration. The straight lines drawn by inspection through the points are shown in fig. 2. Since these lines are not parallel, an arbitrary level of reaction must be chosen at which to make comparisons of potency. The level chosen was a duration of 55 minutes which was produced by 2% procaine, a concentration commonly used. It is notable that the lines for naphthocaine and xylocaine are very flat, i.e. the doses given are above those producing almost maximum duration for these drugs. Consequently relative potencies for these drugs cannot be estimated properly from our data.

### Effect of Epinephrine

Epinephrine 1:100,000 prolonged the anaesthesia of both procaine and dibucaine by about 20%, on the average. Epinephrine 1:20,000, which was tried only with procaine, caused no greater prolongation than 1:100,000. The very slight effect of epinephrine in this situation is hard to explain but it was not entirely unexpected because it was the clinical impression of our colleagues, Drs. A.J. Elliot and C.McCulloch, that the presence or absence of epinephrine made little difference to the duration of retrobulbar blocks in humans.







### Relative Ratings

Table III gives the toxicity to skin of various drugs relative to procaine, as shown by the two different criteria. Also shown are the relative potencies, and in the last two columns the relative ratings calculated from the two different estimates of relative toxicity. Of the three drugs for which complete data were obtained only one, viz dibucaine, showed a relative rating which was significantly greater than that of procaine.

### Estimate of Errors

Each relative potency or relative toxicity contains the errors of two experimental measurements. Consequently each relative rating contains four experimental errors, and a comparison of two relative ratings contains eight experimental errors. We have estimated that a relative rating must be greater than 1.9 or less than 0.52 for the drug to be considered significantly different from procaine, i.e. so that the probability of chance occurrence is less than 1 in 20. Two relative ratings must differ even more to be significantly different, namely by a factor of 2.7. These errors are larger than those computed for relative ratings by Hamilton, Westfall and Ferguson (1), partly because durations of anaesthesia were less uniform for retrobulbar block than for intradermal injection and partly because the error of the intradermal toxicity measurement was even larger and was not evaluated in the earlier paper.

### Discussion

Our results confirm those of Hamilton, Westfall and Ferguson (1) in showing that local anaesthetics have a much wider range of variation in tissue toxicity than in systemic toxicity and hence that tissue toxicity should be used in relative ratings for limited regional effects. Our results differ, however, in finding that Butethamine (Monocaine) is more toxic than the estimate given in the previous paper. Our revised estimate is more in accord with those of other investigators (4).

It is apparent that different estimates of tissue toxicity may be obtained depending on whether erythema or petechial haemorrhage is used as the criterion. In one case (Naphthocaine) this difference appears to be much greater than can be attributed to chance. It is possible that the erythema may be influenced by the vasodilator or constrictor properties of the drug which may not be properly classed as irritant or toxic. This might explain why procaine, a dilator drug, caused such a large area of erythema before it produced petechiae. There is reason from our data to believe that the T.C. 50 (concentration at which petechial damage appears in half the animals) could be measured more accurately than the concentration corresponding to any diameter of erythema. It would probably be a more relevant measure of skin toxicity.







It is important to remember that relative ratings measured on animals may not represent relative ratings for humans. Species differences in relative potencies and toxicities have been reported for many drugs. Until Relative Ratings are measured on humans in some such manner as described above, we will not have quantitative comparisons which are valid for humans.

### Summary and Conclusions

1. Toxicity of nine local anaesthetics for the skin of guinea pigs has been investigated by intradermal injection of various concentrations. Two criteria of toxicity were compared and toxicities relative to procaine were calculated by both methods.
2. Potencies for regional nerve block have been measured for 6 anaesthetics by retro-ocular injection in guinea pigs. Potencies relative to procaine have been calculated.
3. Relative Ratings compared to procaine were calculated by dividing the Relative Potency by the Relative Toxicity for four drugs. Limits of confidence of such ratings ( $p = 0.95$ ) are estimated as +90%, -48%. Dibucaine has a significantly higher rating than the other drugs studied.
4. Epinephrine 1:100,000 had remarkably little effect in prolonging the action of procaine, dibucaine and xylocaine, in this preparation. A concentration of 1:20,000 was no better.

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"B-6"

TABLE I

INTRADERMAL TOXICITY

Average Diameters of Hyperemic Areas (mm.  $\pm$  S.E.)

| Drugs                             | Concentrations % |                  |                  |                  |                   |                   |                   | T.C.50<br>% |
|-----------------------------------|------------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------|
|                                   | 0.05             | 0.1              | 0.25             | 0.5              | 1.0               | 2.0               | 5.0               |             |
| Procaine<br>HCl                   |                  |                  |                  |                  | 4.0<br>$\pm 0.7$  | 5.7<br>$\pm 0.3$  | 7.2<br>$\pm 0.5$  | 5.0         |
| Cocaine<br>HCl                    |                  | 3.4<br>$\pm 0.5$ | 4.7<br>$\pm 0.3$ | 5.7<br>$\pm 0.8$ | 5.0<br>$\pm 0.7$  | 6.7<br>$\pm 1.3$  |                   | 1.6         |
| Tetracaine<br>HCl<br>(Pontocaine) | 3.2<br>$\pm 0.2$ | 5.2<br>$\pm 0.2$ | 7.0<br>$\pm 0.5$ | 8.5<br>$\pm 0.2$ | 10.2<br>$\pm 0.3$ |                   |                   | 0.2         |
| Dibucaïne<br>HCl<br>(Nupercaine)  | 5.2<br>$\pm 0.7$ | 5.7<br>$\pm 0.8$ | 7.2<br>$\pm 0.3$ | 7.3<br>$\pm 0.3$ | 11.0<br>$\pm 0.7$ |                   |                   | 0.4         |
| Butethamine<br>HCl<br>(Monocaine) |                  |                  | 3.7<br>$\pm 0.4$ | 4.0<br>$\pm 0.4$ | 5.0<br>$\pm 0.7$  | 7.2<br>$\pm 1.0$  |                   | 3.0         |
| Piperocaine<br>HCl<br>(Metycaine) |                  |                  |                  | 3.7<br>$\pm 0.2$ | 5.0<br>$\pm 0.5$  | 8.0<br>$\pm 0.7$  | 10.5<br>$\pm 0.7$ | 4.0         |
| Butacaine<br>Sulfate              |                  | 3.2<br>$\pm 0.3$ | 3.5<br>$\pm 0.5$ | 5.5<br>$\pm 0.7$ | 8.5<br>$\pm 0.2$  | 10.7<br>$\pm 0.5$ |                   | 0.8         |
| Octocaine<br>HCl                  | 2.2<br>$\pm 0.2$ | 4.0<br>$\pm 0.8$ | 7.5<br>$\pm 0.5$ | 8.3<br>$\pm 0.4$ | 12.2<br>$\pm 0.7$ |                   |                   | 0.2         |
| Naphthocaine<br>HCl               |                  | 3.5<br>$\pm 0.7$ | 3.7<br>$\pm 0.8$ | 4.5<br>$\pm 0.7$ | 6.2<br>$\pm 0.5$  | 6.4<br>$\pm 0.8$  |                   | 0.8         |

Each figure is the average of four experiments.







"B-7"

TABLE II

RETROBULBAR BLOCK

Average Duration of Loss of Corneal Reflex (Minutes  $\pm$  S.E.)

| Drugs                                             | Concentrations % |              |              |              |
|---------------------------------------------------|------------------|--------------|--------------|--------------|
|                                                   | 0.5              | 1.0          | 1.5          | 2.0          |
| Procaine<br>HCl<br>(Metycaine)                    | 26.4<br>+2.7     | 42.0<br>+3.1 | 55.8<br>+2.1 | 51.4<br>+1.5 |
| Piperocaine<br>HCl<br>(Metycaine)                 | 33.0<br>+4.2     | 48.6<br>+4.2 | 64.2<br>+2.1 | 71.4<br>+2.1 |
| Naphthocaine<br>HCl                               | 54.6<br>+2.7     | 51.8<br>+4.6 | 56.6<br>+7.2 | 57.0<br>+3.6 |
| Xylocaine<br>HCl                                  | 31.4<br>+0.6     | 37.4<br>+1.5 | 36.2<br>+1.8 | 33.8<br>+2.1 |
| Xylocaine<br>HCl<br>with Epinephrine<br>1:100,000 | 35.0<br>+1.5     | 34.2<br>+3.2 |              | 45.2<br>+4.6 |

Concentrations %

|                                   | 0.1          | 0.25         | 0.5           | 0.75          | 1.0          |
|-----------------------------------|--------------|--------------|---------------|---------------|--------------|
| Tetracaine<br>HCl<br>(Pontocaine) | 44.6<br>+2.8 | 80.2<br>+1.8 | 126.2<br>+9.0 | 133.4<br>+3.9 | 201.8<br>+14 |

Concentrations %

|                                  | 0.01         | 0.025        | 0.05         | 0.1           |
|----------------------------------|--------------|--------------|--------------|---------------|
| Dibucaine<br>HCl<br>(Nupercaine) | 43.6<br>+3.6 | 75.0<br>+5.4 | 99.8<br>+6.6 | 127.0<br>+3.0 |

Each figure is the average of five experiments.







"B-8"

TABLE III

RELATIVE TOXICITIES, POTENCIES AND RATINGS (PROCAINE =1)

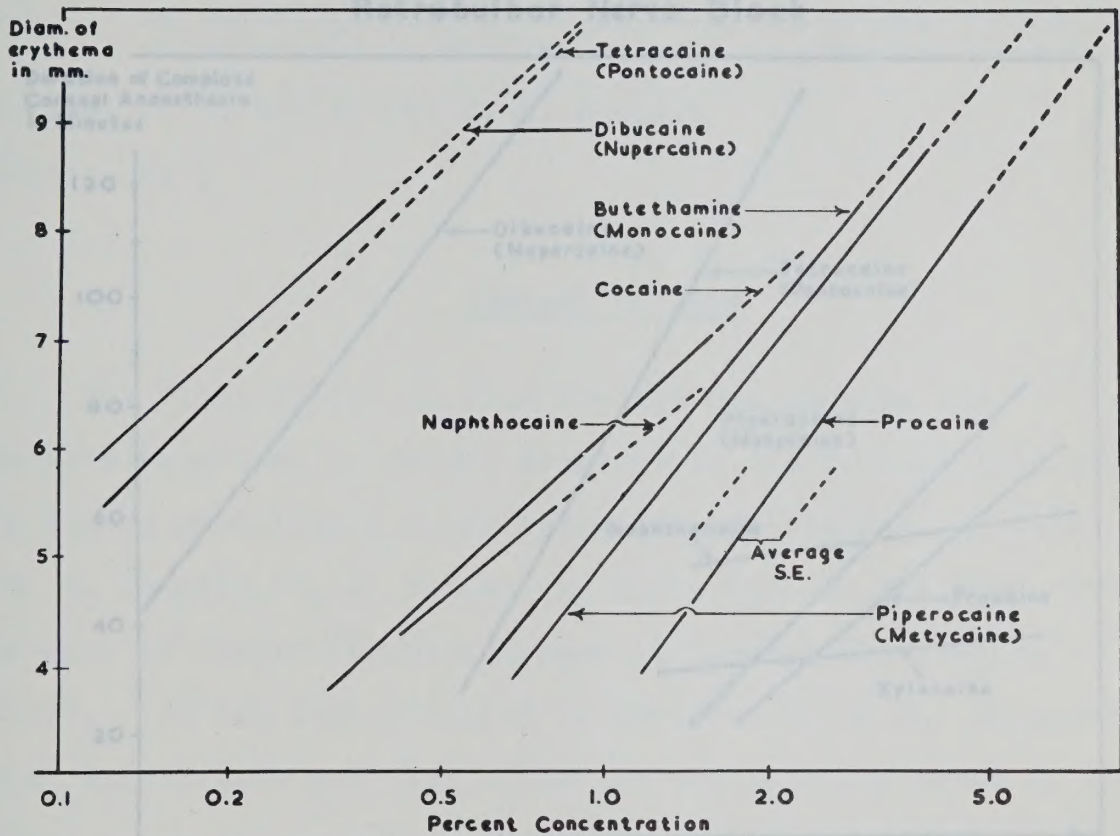
|                                   | Rel.Tox<br>by<br>T.C.50 | Rel.Tox<br>by 6mm.<br>Eryth. | Rel.Pot.<br>by<br>R.B.Block | Relative<br>Rating<br>c/a | Relative<br>Rating<br>c/b |
|-----------------------------------|-------------------------|------------------------------|-----------------------------|---------------------------|---------------------------|
|                                   | 'a'                     | 'b'                          | 'c'                         |                           |                           |
| Piperocaine<br>HCl<br>(Metycaine) | 1.25                    | 1.5                          | 1.7                         | 1.4                       | 1.1                       |
| Tetracaine<br>HCl<br>(Pontocaine) | 25                      | 15                           | 14.3                        | 0.6                       | 0.95                      |
| Dibucaïne<br>HCl<br>(Nupercaine)  | 12.5                    | 17                           | 133.0                       | 10.6                      | 7.8                       |
| Naphthocaine<br>HCl               | 6.2                     | 2.1                          |                             |                           |                           |
| Cocaine                           | 3.2                     | 2.4                          |                             |                           |                           |







## Intradermal Toxicity









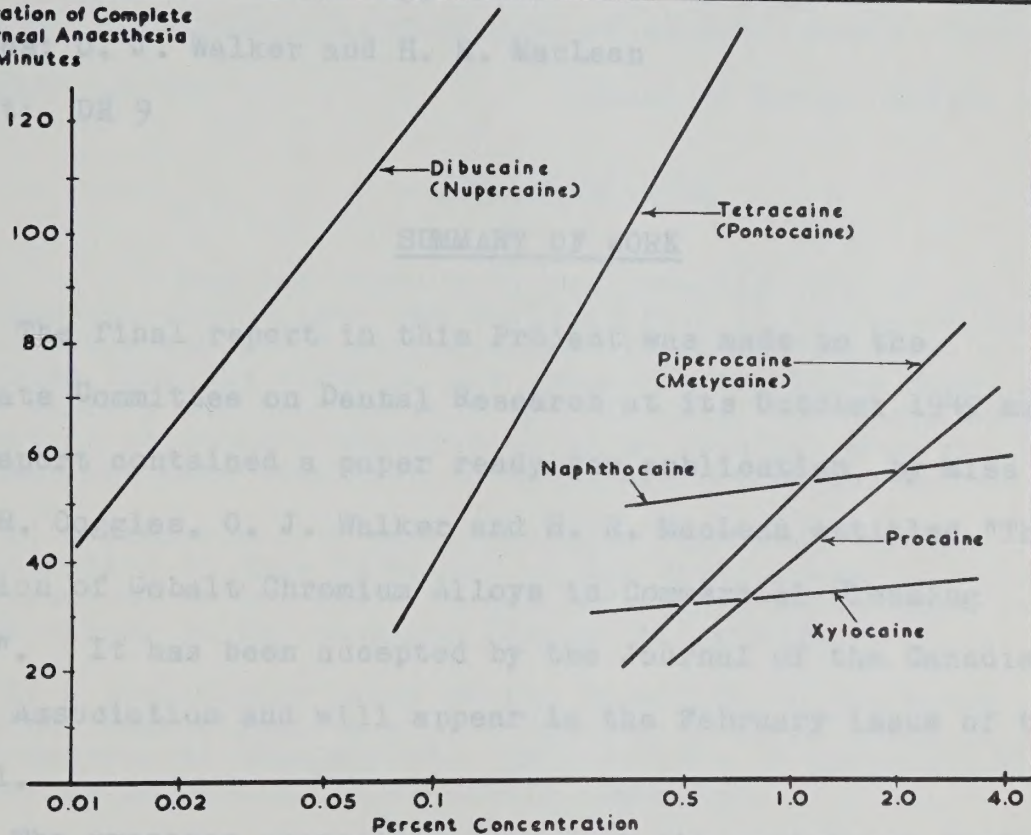
# APPENDIX "C"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

### Retrobulbar Nerve Block

Duration of Complete  
Corneal Anaesthesia  
in Minutes



O. J. Walker, Head  
Department of Ophthalmology  
University of Alberta,  
Edmonton, Alta.

January 20, 1950.







APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry.

Grantees: O. J. Walker and H. R. MacLean

Project: DR 9

SUMMARY OF WORK

The final report in this Project was made to the Associate Committee on Dental Research at its October 1949 meeting. This report contained a paper ready for publication, by Miss D. E. R. Coggles, O. J. Walker and H. R. MacLean entitled "The Corrosion of Cobalt Chromium Alloys in Commercial Cleaning Agents". It has been accepted by the Journal of the Canadian Dental Association and will appear in the February issue of the Journal.

The grantees appreciate very much the assistance rendered to them by the Associate Committee.

January 20, 1950.

O. J. Walker, Head  
Department of Chemistry  
University of Alberta,  
Edmonton, Alta.







## APPENDIX "D"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon.

Grantee: C. P. Leblond, Professor of Anatomy, McGill University.

Project: DR 23 Amount of Grant: \$2800.

#### SUMMARY OF WORK

Besides continuing our investigation of the entry of radio-phosphorus into the teeth of rats of different ages, a considerable amount of work was done in young hamsters, as it was found that the width of the enamel in the tooth germs of these animals was much greater than in rats, and therefore, the deposition of radio-phosphorus could be examined more easily. Two publications were completed in the course of this year:

1) A comprehensive paper entitled "Radio-autographic Visualization of Bone formation in the Rat", a copy of which was forwarded to Dr. Ellis in October, 1949. This paper is due to appear in the American Journal of Anatomy.

2) A preliminary paper on the formation of the tooth, entitled "Mineralization of the Growing Tooth as shown by Radio-phosphorus Autographs". This article is enclosed. The permission to publish it is hereby requested.

Our work may be briefly summarized as follows. When small doses of radio-phosphorus are administered to an animal, they indicate the fate of phosphorus in the body of this animal. When this phosphorus is localized in histological slides by the autographic method, soon after injection, some degree of reaction is found throughout bones and growing teeth. These diffuse reactions may be explained through a process of "exchange" between the radio-phosphorus of the extracellular fluid and the surface phosphate groups of the bone and tooth crystals. Within a few days after injection of radio-phosphorus, there was a gradual decrease and disappearance of these diffuse reactions. This phenomenon is explained by an "exchange" in the reverse direction, as the radio-phosphate of the crystal surfaces is replaced by some of the circulating phosphate which, by that time, no longer contains significant amounts of radioactivity. It is believed, however, that this interesting phenomenon does not play an important role in physiology.



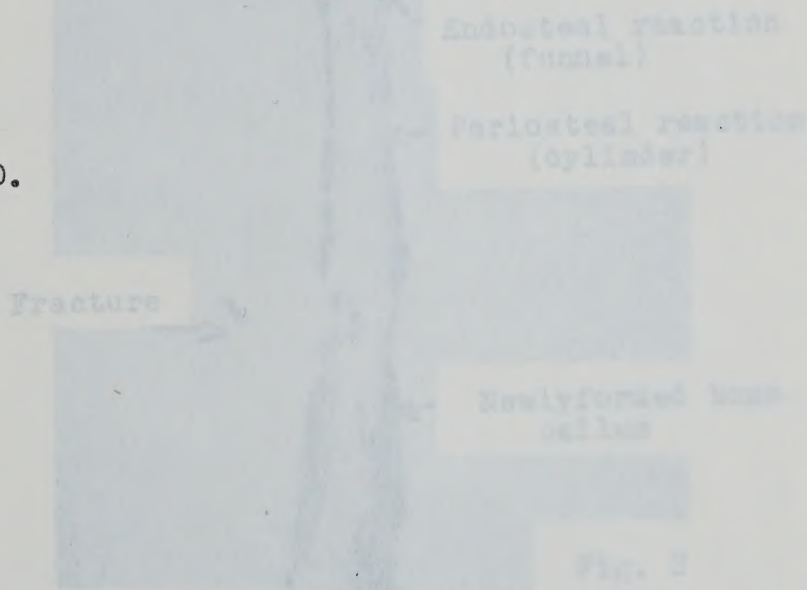




Of extreme importance, on the other hand, are the reactions restricted to definite parts of bones and teeth, since these limited reactions persist for long periods of time after injection and, indeed, reveal the deposition of new bone or tooth tissue. Thus, soon after injection, the area of the dentin close to the odontoblasts shows a dark line indicating the location of the dentin formed in the few hours following the injection of radio-phosphorus. A few days later, the reaction line is in the substance of the dentin away from the odontoblasts, as may be seen in Fig. 1. Presumably, the light area separating the reaction line from the odontoblasts at that time corresponds to the dentin formed after the period when the tissue fluids contained a large amount of radio-phosphorus. Similar reaction lines were encountered in the bones, as may be seen in the paper previously forwarded to Dr. Ellis. The enclosed picture of a longitudinal section of a long bone (Fig. 2), was obtained in an animal sacrificed four hours after an injection of radio-phosphorus, given four days after the fracture of one wall of the tibia. Reactions typical of normal bone, may be seen in the upper part of the picture and, typical of fractured areas, in the lower part.

It is hoped that these results will offer a new approach to physiological and pathological problems of bones and teeth.

January 11, 1950.









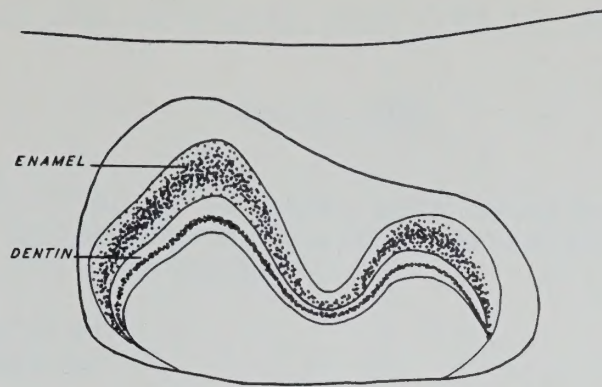


Fig. 1

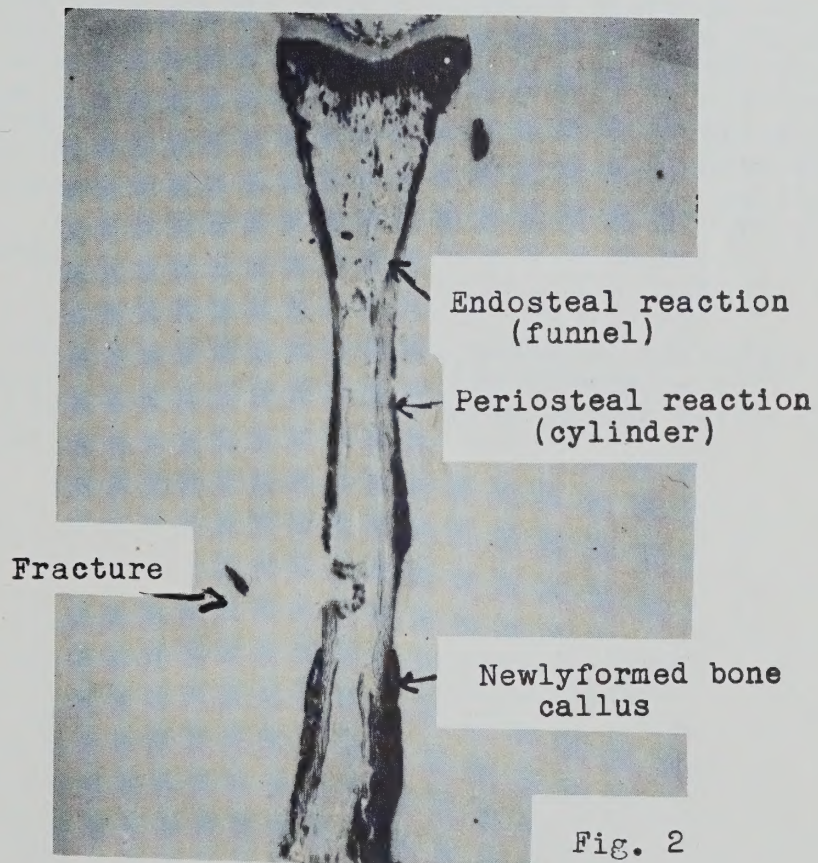


Fig. 2







## APPENDIX "E"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: A histological study of tooth germ development, both intra- and extra-alveolar with a survey of the pathological changes in the developing tooth.

Grantee: Dr. J. W. Abbiss  
Dr. A. G. Nutlay

Project: DR 27

#### SUMMARY OF WORK

The plan outlined in the interim report rendered six months ago has proceeded. As yet the work has consisted mainly of a study of the embryology and histology of the developing human tooth. The collection of material in this connection has proceeded, and serial sections of the jaws of fetuses at various stages of development and of young infants, have now been made. (Previously this type of material was not available at this centre). In addition a collection of dental pathological specimens has commenced.

As the collection of pathological material increases with time, findings will be correlated with the knowledge acquired by the study of the large amount of apparently normal material which is being accumulated.

J. W. Abbiss;  
A. G. Nutlay.







SUPPLEMENT TO APPENDIX "E"

February 25, 1950.

Report to the  
Associate Committee on Dental Research,  
Concerning the work of Dr. J.W. Abbiss and Dr. A.G. Nutlay  
under DR 27, Halifax, N.S.

Mister Chairman, Gentlemen,

On behalf of the Halifax Grantees of the National Research Council I extend our warm greetings to this Assembly. Also I should like to let you know how much we appreciate the support which was given to us by the Associate Committee on Dental Research at a time when our working plan has started to assume the pattern of a definite scientific composition. Our plan of study bears the following title: A histological study of tooth germ development both intra-and extra-alveolar with a survey of pathological changes in the developing tooth.

You may see clearly from this title that the subject of our observation is a field of vast extent. Theoretically it comprises the structural elements of the growing and developing tooth germ, whether it spends its life cycle intra or extra-alveolar in the jaws; it involves the study of the neighbouring organs of the growing tooth germ which might be interdependent upon each other showing their topographical relationship in a structural modification. The observation of these features so characteristic of a complex mechanism is a part only in our programme which includes also the study of pathological changes to be found around and in the tissues of the developing tooth.

The selected study material is obtained through the courtesy of the Nova Scotia Pathological Institute in Halifax, N.S.

We divided our working programme into two parts. In the first part we are preparing serial sections of tooth buds in the early, embryonic stage of their existence. They are surrounded by embryonic, connective tissues, or coarse, primitive bone formations. With reference to this embryonic tooth development we have collected 8 specimens. They are in the cyto- and morphodifferentiation period, and surrounded in three sides by osteoid, primitive, connective tissue. Their enamel organ is still connected with the oral epithelium through the dental lamina and the numerous mitoses in and around the enamel organ signify a very active cellular response. In this stage of tooth growth and development it is of paramount importance to retain the exact topographical relationship of the developing odontogenic and non odontogenic cells. A true







## SUPPLEMENT TO APPENDIX "E"

- 2 -

understanding of their mutual effect to each other's course of regular development might open the door to investigate the cause of many pathological entities occurring so often in these areas. The structural order of cellular elements of extra-alveolar developing tooth germs will form the second part of our working programme. The purpose of our study of tooth growth and development as they are represented to us by microscopic slides, might be schematised advantageously through chronological methods of procedure. The reason of summarising the purpose of study in points, following the great American Research Workers, is as follows: 1)-st. to test the validity of each theory of tooth development, whether it has been advanced through clinical or experimental findings, by our histological observations. 2)-nd to ferret out those facts of development which could withstand this scrutiny. 3)-rd to integrate these facts with the hope of effecting a better understanding of the mechanism of tooth development. 4)-th to suggest on this basis further experimental studies on the problem. In our particular case we may append a further point no 5 to be able to discern in the further course of tooth development the effect from the cause. This point no. 5 will guide the proceedings in the second part of our working programme. Theories propounded and statements asserted on our subject must be approached in the light of histological evidence for and against their thesis.

The problem which might arise out of intra-and extra-alveolar tooth germ development could be expressed on the basis of the following question: Whereas the periodontal membrane and the alveolar bone are developed from the dental sac (dental follicle) in an intra-alveolar tooth germ development, what would be the function of the dental sac in an extra-alveolar tooth germ development, where no periodontal fibers and alveolar bones develop. There is no literature to the effect that the periosteum of the surrounding bone would act like a periodontal membrane, and furthermore they do not show the histology of bundle bone.

Another point which strikes the observer in this connection might be brought up as follows: "The function of the cementum - and I quote from Noyes-Schour-Noyes" Text-book of Dental Histology and Embryology, 1944 - is to attach the tooth to the connective tissue fibers of the periodontal membrane. The primary purpose of cementum is to support the tooth as a whole. The presence and a probable influence of Hertwig's epithelial sheath on the formation of cementum presents a problem. "As connective tissue cells especially in this stage of tooth growth and development are not supposed to be capable of creating differentiated tissues as the periodontal fibers, alveolar bones, and cementum; might it not be logical to think that these beforehand mentioned connective tissue organs were built at least through stimulation of Hertwig's epithelial sheath?







APPENDIX "F"

SUPPLEMENT TO APPENDIX "E"

REPORT TO THE ASSOCIATE CH - 3 -

The eruption of teeth, though it does not belong to our subject of study, is another process which because of its mechanism should be mentioned briefly in connection with our working programme. When the tooth germ migrates toward the oral epithelium in the second stage of eruption, primitive periodontal fibers are to be seen extending from the dental sac, at this time developed to the alveolar bone, toward Tomes granular dentine layer. Thus a pre-condition of eruption would be the existence of the alveolar bone. But what about the eruption of impacted displaced teeth which are embedded in a bony crypt histologically different from the alveolar bone? These teeth erupt sometimes in the presence of a full complement of teeth. It is interesting to note that every slight change in the position of teeth is followed by a formation of a new layer of secondary cementum, in order to reattach the fibers of the periodontal membrane in the new positions.

This report of our activities in the present, and of our problems to be dealt with in the coming season, comprises our programme of studies. I have with me some of our slides, representing the histological picture of an embryonic phase of a tooth germ, where the enamel organ is developed intra-alveolar.

Respectfully submitted,

"Dr. J.W. Abbiss,

Dr. A. G. Nutlay"







## APPENDIX "F"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth

Grantee: Dr. C.H. Best

Project: D.R. 22 Amount of Grant: \$2,400.

#### SUMMARY OF WORK

An experimental method has been developed for securing functional reattachment of the supporting structures of the teeth. Apposition of new cementum on cementum and directly on dentine occurred, attaching the fibres of the periodontal connective tissue to the denuded tooth surface. Investigations are being carried on for the purpose of studying the physiological principles involved in the reattachment process.

An experimental method for securing regeneration of alveolar bone has been devised and is being studied. It is hoped that an interim report on the findings can be made as soon as this phase of the work is completed. The experimental method of alveolar bone regeneration permits study of the underlying factors involved.

The animals used in the above studies are dogs. Observations on the living animal and histological sections are the criteria employed.







## APPENDIX "F"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

#### STUDIES IN THE REGENERATION AND REATTACHMENT OF SUPPORTING STRUCTURES OF THE TEETH NO. 1. SOFT TISSUE REATTACHMENT

by

W.J. Linghorne, D.D.S., D.C. O'Connell, M.S.A.,  
Banting and Best Department of Medical Research,  
University of Toronto

#### Introduction

A survey of the literature reveals a sharp cleavage of opinion regarding the possibility of functional regeneration and reattachment of the supporting tissues of the teeth once these tissues have been severed or lost during the course of periodontal disease. This subject has been extensively discussed in the literature. Excellent reviews are to be found in Goldman's book (1), and more recently in papers by Orban(2) and Morris (3).

The experimental work in the field has been mainly of two types. In the first type, studies have been made of repair following the destruction of sections of the supporting structures remote from and not involving the gingival crevice. (Beckwith et al., 4; Beube, 5). As these experimental lesions did not involve the gingival crevice it has been questioned whether the results of such work may be accepted as evidence of the possibility of regeneration and attachment following pocket formation in periodontal disease. The second type has been the study of repair following the creation of artificial pockets produced by surgical separation of the soft tissues from the tooth, (Skillen and Lundquist (6)). They found that only little connective tissue reattachment occurred following such procedure. Their conclusion was that the epithelium seemed to proliferate and cover the denuded tissue so rapidly that all further connective tissue reattachment was prevented.

As is brought out in the various reviews, there are many complicating factors in the clinical periodontal problem of the regeneration and reattachment of the supporting structures of the teeth. It seemed advisable, therefore, to study the problem under less complex conditions, i.e. by bringing about the loss of supporting structure in healthy supporting tissue,



by surgical means: While it is recognized that this experimental situation differs in some respects from the clinical, it appeared more favourable for the investigation of some of the fundamental aspects of this problem, i.e. the potentiality of the supporting structures for regeneration and reattachment, also the underlying factors and principles involved in the process.

This paper deals with a study of the reattachment to the tooth of the soft tissues. A report on the regeneration of alveolar bone will be made later. Connective tissue reattachment of the soft tissues to the tooth by means of a deposition of new cementum was repeatedly attained in the present investigation.

### Procedure

The experimental animals were dogs with healthy gingival tissues. Only the four canine teeth were used. By means of a disc stone, a groove was cut into the tooth approximately 1 mm. from the gingival margin. (Fig. 3). This groove served as a point of reference from which the level of the gingival sulcus or pocket could be measured. Thus it was possible to obtain accurate data on the gingival recession and of reattachment of the surgical flap. A gingival flap was made exposing the overlying alveolar bone, care being taken to detach the complete periosteum including the inner or cambium layer. Then a section of bone and periodontal membrane approximately 7 x 6 mm. was removed exposing the tooth. The flap thus detached measured approximately 9 mm. from the bottom of the gingival sulcus or 12 mm. from the groove in the tooth. In some cases the tooth was scaled, in others no scaling was done. The flap was sutured into place. Following surgery, in the greater apical part of the flap the disposition of the tissues from within outwards was as follows: denuded or scaled tooth surface, inner layer of periosteum, outer layer of periosteum, connective tissue of the gingiva, oral epithelium. Figures 1 and 2 are sketches illustrating the disposition of the tissues before and after the surgical procedure.

By means of a probe graduated in millimetres, the level of the bottom of the gingival sulcus was measured from the gingival mark on the tooth before operation and again at the end of the healing period. Twenty animals were sacrificed at various intervals from 5 days to 1 year and blocks were removed for sectioning. Paraffin sections were made after fixation in 10% formal-saline and decalcification in 5% formal-nitric acid, and were stained with haematoxylin and eosin.



### Observations on the living animal

In the upper teeth when measured by a probe, 6 to 8 mm. of reattachment appeared to have occurred in all but one instance, when the healing period exceeded 18 days. Before that period had elapsed, sufficient pressure could not be exerted on the probe to determine whether attachment had taken place, without the risk of tearing the healing wound. After the 18th day the wound appeared to have healed and the gingiva had resumed a normal appearance (Fig. 3). Usually 1 to 3 mm. of recession were found.

On the other hand in the lower canines, only 1 to 3 mm. of attachment appears to have occurred. Often there was considerable recession over the area, the pocket depth being related to the amount of recession. The gingiva did not regain the normal appearance as did the gingiva in the uppers.

### Histologic observations

The histologic examination of the specimens permitted the differentiation of two groups of cases. One, represented almost solely in the lower jaw, ended with little reattachment to the tooth and the formation of a true epithelialized pocket. In addition, gingival recession occurred in some of the teeth. The second group, those in the upper jaw, can be considered as successful connective tissue attachment of the detached gingiva to the exposed tooth surface. Some regeneration of the removed alveolar bone could be observed in most of the experimental animals where attachment of the flap occurred.

Figure 4 is a photomicrograph of a lower canine with a 14 day healing period. No cementum is apparent on the surface of the tooth from which the supporting tissues have been detached; also there is some loss of dentine. The possibility must be considered that at least part of this lost tooth structure may have been removed at operation together with the bone and periodontal membrane or by scaling. The pocket is inadequately covered by epithelium, little epithelium being seen over the lower half of the pocket. However what appears to be an epithelial attachment (high-power insert) was observed in a field about 1 mm. from the base of the artificial pocket which was approximately 10 mm. in depth. The connective tissue is inflamed with an increased cellular infiltration around some hairs which were found in many of the lowers. In contrast only a few hairs were found in the uppers on microscopic examination. The presence of these hairs in the tissue will be discussed later in this paper.



In the upper jaw, connective tissue attachment of the flap to the tooth, by means of the deposition of new cementum, occurred over approximately  $\frac{3}{4}$  of the denuded tooth surface. Usually, 2 to 3 mm. of recession took place.

Figure 5 is a photomicrograph of an upper canine showing attachment after a 49 day healing period. Figures 6, 7 and 8 are higher magnifications of selected fields from Fig. 5. Figure 6 shows the junction of the old and new attachments. Above the junction, in the zone of new attachment, all of the cementum and some of the dentine have been lost. New cellular cementum has been laid down on the dentine but has been pulled away. This separation is considered to be artefact (see description of Fig. 7). The connective tissue fibers appear to be attached in the usual manner but run in a direction parallel to the tooth rather than in the characteristic oblique or horizontal direction. This was the arrangement of the connective tissue fibers wherever the reattachment had taken place. Note the absence of any appreciable cellular infiltration.

Figure 7 is a view of the same section about  $\frac{1}{2}$  mm. gingivally to that of Fig. 6. Both attached cementum and an extension of the detached cementum shown in Figure 6 can be seen. In this photograph it is observed that at least some of the loss of tooth structure is due to resorption. Note the well differentiated cementoblast layer (insert is higher magnification at point X). In this section the parallel arrangement of the connective tissue fibers described above is quite distinct.

Fig. 8 is a view of the gingival sulcus of the specimen shown in Fig. 5. It is evident that some dentine had been resorbed, but so far much less cemental apposition has occurred than was shown in Figs. 6 & 7. Note that the epithelium had not proliferated rootwise as was the case with the lowers. There is little cellular infiltration.

Sections of all cases up to the 19th day show a separation of the soft tissues from the tooth. This is believed to be artefact, caused either in the trimming of the block or during the preparation of the sections; the early union between the tooth and soft tissues being weak. From the 19th to the 35th day of healing, considerable artefact was encountered but evidence of reattachment was found in many of the sections which suggested that in the living animal the tissues were attached. This is illustrated in Figs. 9 & 10. Fig. 9 is a photomicrograph of a field of a section from an upper canine, 19 day healing period, in which it is apparent that some of the connective tissue fibers are attached, even though in most of the section the soft tissues were separated from the tooth. This specimen shows the loss of cementum and some dentine from the tooth and the parallel arrangement of the connective tissue fibers, described in previous slides.



Some cellular infiltration is still present. Fig. 10 illustrates a type of separation that is often encountered. The attached tags of tissue suggest that the connective tissue was torn. This view also shows early evidence of cemental apposition on resorbed dentine. However in Fig. 11, which shows a field of another section from the same tooth, there is a considerable thickness of new cellular cementum laid down on resorbed dentine. It is evident that activity in resorption and apposition varies in different areas along the tooth. Indeed, in some of the specimens where attachment took place, areas were noted where little if any evidence of resorption or apposition was apparent. (Fig. 8). Again, other areas were seen where the tissues were separated from the tooth, in which little if any resorption, apposition or other evidences of reattachment could be found. Fig. 12: A view of an upper canine, 49 day healing period, shows such an area. The resorbed and attached area (towards the top of the photomicrograph) lies gingivally to the area described. However, under high power, cells were observed which appeared to have differentiated into cementoblasts. They were rounded up, stained deeper and were related to a deeper staining inter-cellular substance. They appeared much more widely scattered than the cementoblasts shown in Fig. 7. If these cells are cementoblasts, attachment will probably occur later, if it has not already done so. The lack of cellular infiltration suggests that even if attachment has not taken place yet, this area does not communicate with the gingival crevice.

One of the most interesting findings in this study is the behaviour of the epithelium. Fig. 13 is a view of the crevice epithelium and adjacent gingiva of an upper canine, 22nd day of healing, where on microscopic examination the soft tissues were found to be separated from the tooth. We have stated that such separations are believed to be artefact. The fact that the epithelium did not proliferate rootwise in this specimen suggests that in the living animal attachment had taken place. Also of interest in this regard is the relative lack of cellular infiltration into the connective tissue of the gingiva.

What appears to be a regeneration of alveolar bone was observed in many of the sections where attachment took place. Fig. 14: a view of an upper canine, 22 day healing period, is a typical example. The characteristic loss of cementum and some dentine, apposition of new cementum, parallel direction of the fibers and the newly differentiated cementoblasts can be seen. The space between the new cementum and the dentine is believed to be artefact.

## Discussion

Although the technique for detachment of the periosteum from the alveolar bone and its replacement over the denuded tooth surface was developed because of the alleged osteogenic potency of the periosteum and its property of acting as a limiting



membrane, the results of the experiment do not preclude a different interpretation. It is possible that the loose connective tissue of the cambium layer is to be preferred to the dense connective tissue of the gingiva for reattachment. This does not necessarily mean that under suitable conditions the required cellular differentiation for attachment cannot take place when healing occurs by granulation tissue arising from the connective tissue of the gingiva. Also in comparing our results with those reported by Skillen and Lundquist, the possibility must be considered that simply better adaptation of the detached tissues to the tooth may be a factor in the end result.

The result in the lowers, where the prominent feature was the development of a true epithelialized pocket rather than connective tissue attachment, appears similar to the result reported by Skillen and Lundquist. A number of possibilities for failure of attachment have been considered. First it was noted that the periosteum was more difficult to remove from the alveolar bone in the lower canines than in the uppers. Sections of alveolar bone removed after the periosteum had been detached showed that considerably more of the cambium layer remained attached to the bone in the lower than was the case with the upper jaw where little soft tissue could be observed remaining on the bone. Another possible reason for failure was that in many cases hairs were found in the wounds in the lowers, possibly because the dogs rubbed the gingiva with their paws after operation. Only very occasionally was hair seen in the uppers. Another factor might be the higher muscle attachment. Obviously these questions and others brought up in the discussion can only be answered by further work, some of which is now under way.

The variation in activity indicated by the decided difference in the amount of resorption and apposition along the tooth, where reattachment had taken place, is hard to explain. One would not expect an even resorption but the reason for the variation in the amounts of cemental apposition in different positions on the tooth is not understood. In general, the greatest thickness of new cementum was found in the detached area near the base of the wound.

In commenting on the behaviour of the epithelium all that can be stated is that it seemed to proliferate apically when the connective tissue attachment failed but not when it took place. Thus it might be argued that the epithelium has a passive rather than active role in pocket formation; that the apical progression of the epithelium by making the denuded tooth surface an outside surface, may prevent serious resorption of the tooth when for any reason the continuous apposition of cementoid material is interrupted.



In most cases where reattachment had taken place some regeneration of alveolar bone was noted on histologic examination. However in a number of older experimental cases where reattachment of the soft tissues to the tooth appeared in the gross to have occurred, a second gingival flap was made for the purpose of examining the alveolar bone. It was found that there was some slight regeneration of the alveolar bone at the base of the wound; however resorption of the bone at the edges of the wound was noted. Appreciable bone regeneration had not occurred up to one year after operation when examined in the living animal.

The reason only seven lower cuspids as against nineteen uppers were used in this study was, that preliminary studies had already indicated that the upper canines were to be preferred over the lowers for an attachment study.

### Conclusions

1. In dogs, following the surgical destruction of a section of the periodontal membrane and alveolar bone, connective tissue reattachment of the gingival tissues to the tooth by a deposition of new cementum was repeatedly obtained.
2. Uneven resorption of both cementum and dentine takes place prior to cemental apposition.
3. New cementum may be laid down on the old cementum or directly on dentine.
4. In the reattached gingiva, the connective tissue fibers run in a direction parallel to the tooth rather than in the characteristic oblique or horizontal direction.
5. The oral epithelium appears to proliferate downward when reattachment fails but not when it takes place.
6. Some regeneration of alveolar bone occurred.



KEY TO ABBREVIATIONS IN ILLUSTRATIONS

|     |                                                                                        |
|-----|----------------------------------------------------------------------------------------|
| A   | Artefact (Separation believed to have occurred during the preparation of the sections) |
| AB  | Alveolar bone                                                                          |
| B   | Base of pocket                                                                         |
| C   | Cementum                                                                               |
| CB  | Cementoblast                                                                           |
| CC  | Cementocyte                                                                            |
| CE  | Crevice epithelium                                                                     |
| CI  | Cellular infiltration                                                                  |
| CL  | Cambium layer                                                                          |
| CTF | Connective tissue fibers                                                               |
| D   | Dentine                                                                                |
| E   | Epithelium                                                                             |
| EA  | Epithelial attachment                                                                  |
| G   | Groove in tooth                                                                        |
| GT  | Gingival tissues                                                                       |
| IA  | Inactive area described in text                                                        |
| J   | Junction of old and new attachments                                                    |
| NAT | Newly attached tissue                                                                  |
| NC  | New cementum                                                                           |
| OC  | Old cementum                                                                           |
| OD  | Old dentine                                                                            |
| P   | Polymorphonuclear leucocyte                                                            |
| PM  | Periodontal membrane                                                                   |
| R   | Resorption                                                                             |
| RL  | Resorbed dentine with reversed line                                                    |
| RR  | Resorbed and reattached area                                                           |
| SA  | Separation artefact                                                                    |
| X   | Insert                                                                                 |



Legend

Figs. 1 and 2: Illustrations of the disposition of the supporting structures of the teeth before and after surgical procedure.

Fig. 3: Twenty-two day specimen, upper canine. Typical of appearance of gingiva.

Fig. 4: Fourteen day specimen, lower canine where reattachment failed. Note absence of cementum and loss of some dentine.

Fig. 5: Forty-nine day specimen, upper canine, showing reattachment.

Fig. 6: Forty-nine day specimen, higher magnification of Fig. 5 at the junction of old and new attachments. (Area enclosed in circle at 7).

Fig. 7: Forty-nine day specimen, higher magnification of Fig. 5, one-half millimetre gingivally to Fig. 6. (Area enclosed in circle at 7).

Fig. 8: Forty-nine day specimen, higher magnification of Fig. 5 showing Gingival sulcus. (Area enclosed in circle at 8).

Fig. 9: Nineteen-day specimen, upper canine. Note loss of cementum and some dentine, and the parallel arrangement of new connective tissue fibers.

Fig. 10: Twenty-one day specimen, upper canine.

Fig. 11: Twenty-one day specimen, upper canine.

Fig. 12: Forty-nine day specimen, upper canine.

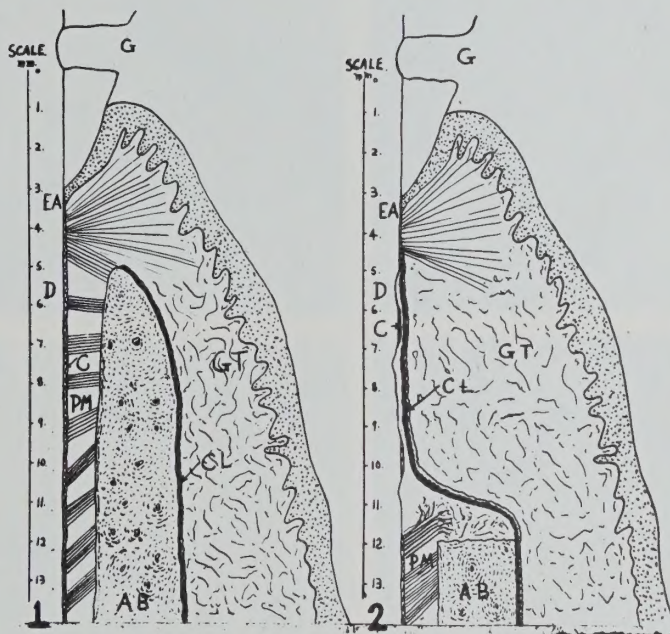
Fig. 13: Twenty-two day specimen, upper canine. Note: The separation artefact was so wide that the tooth could not be shown in the same field at this magnification. Note the relative lack of cellular infiltration.

Fig. 14: Twenty-two day specimen, upper canine.





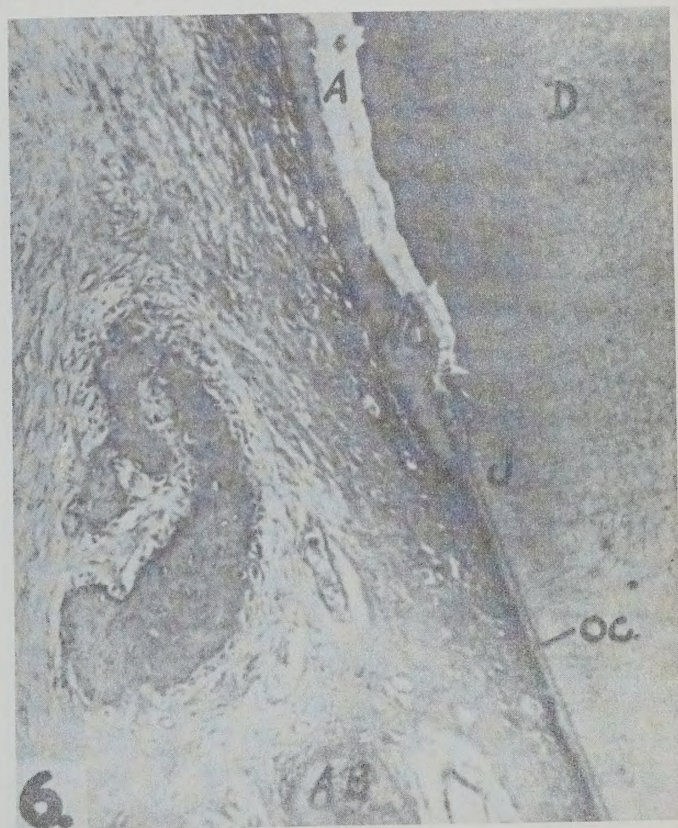
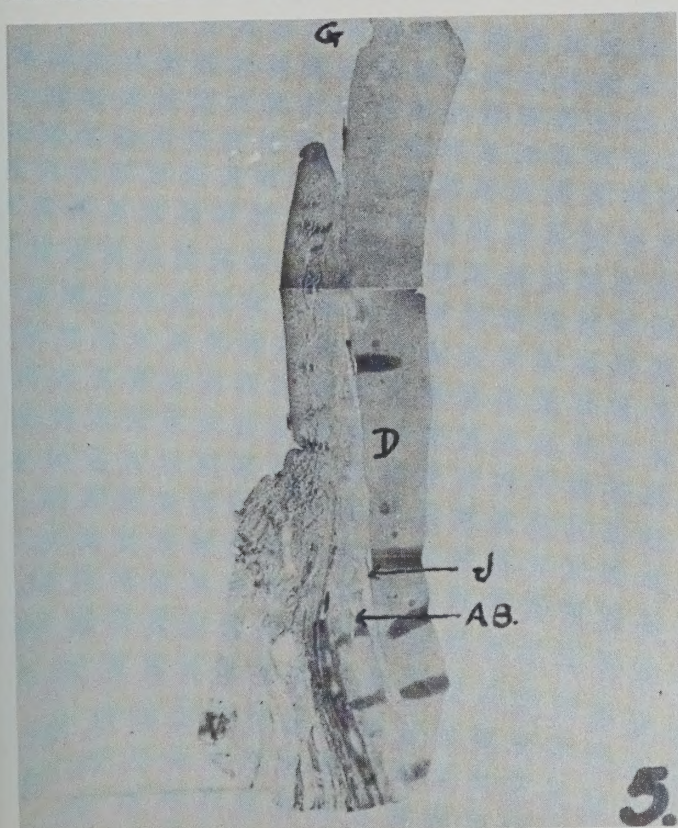
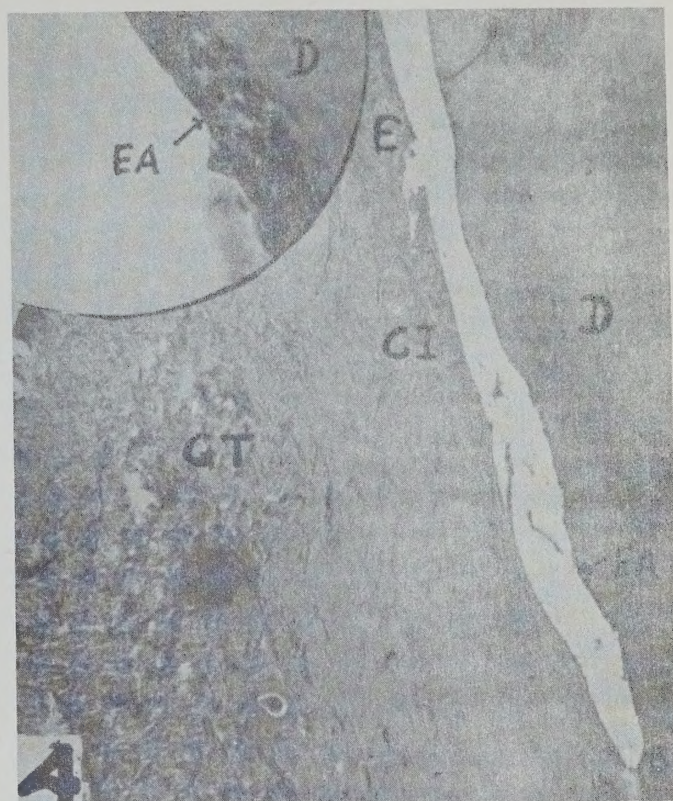
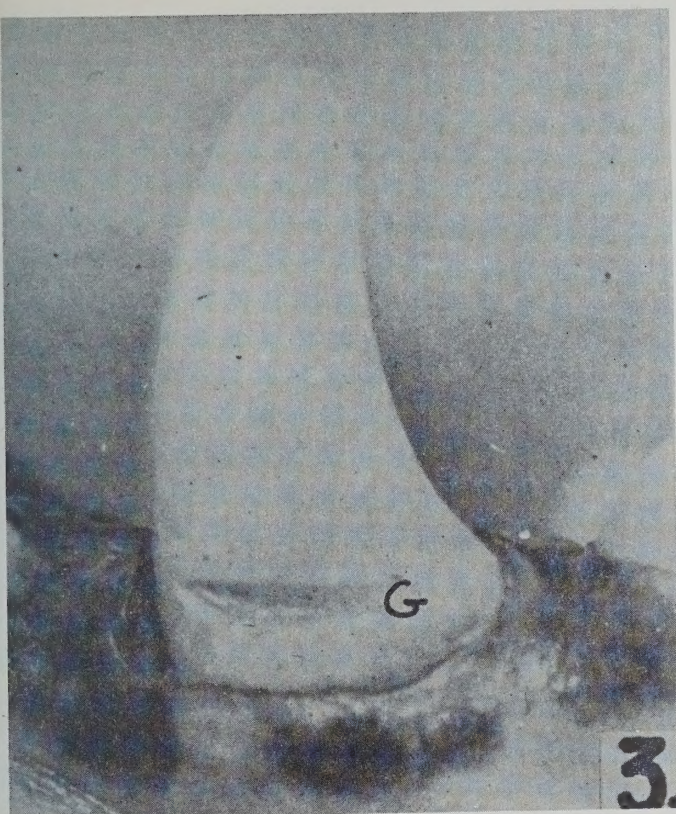








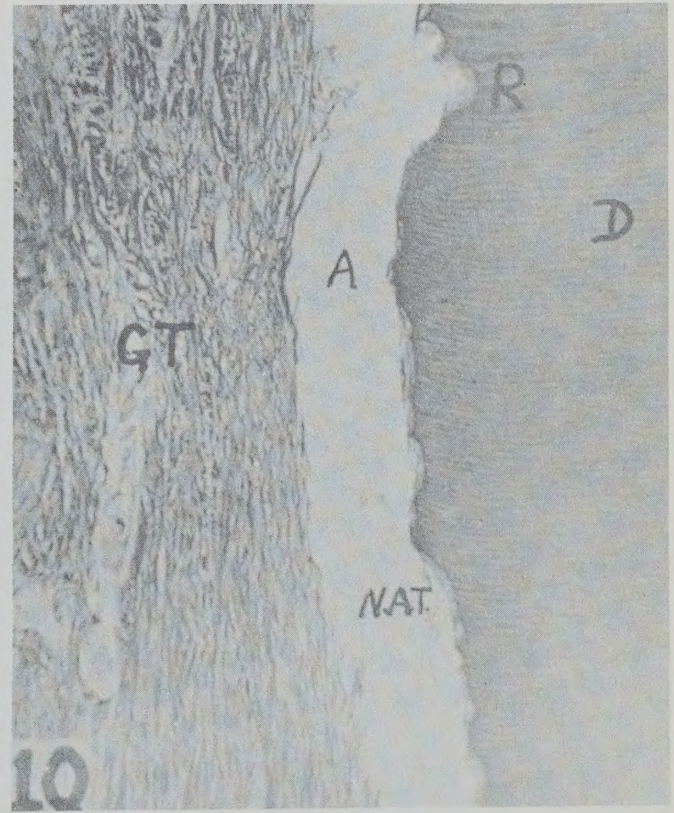
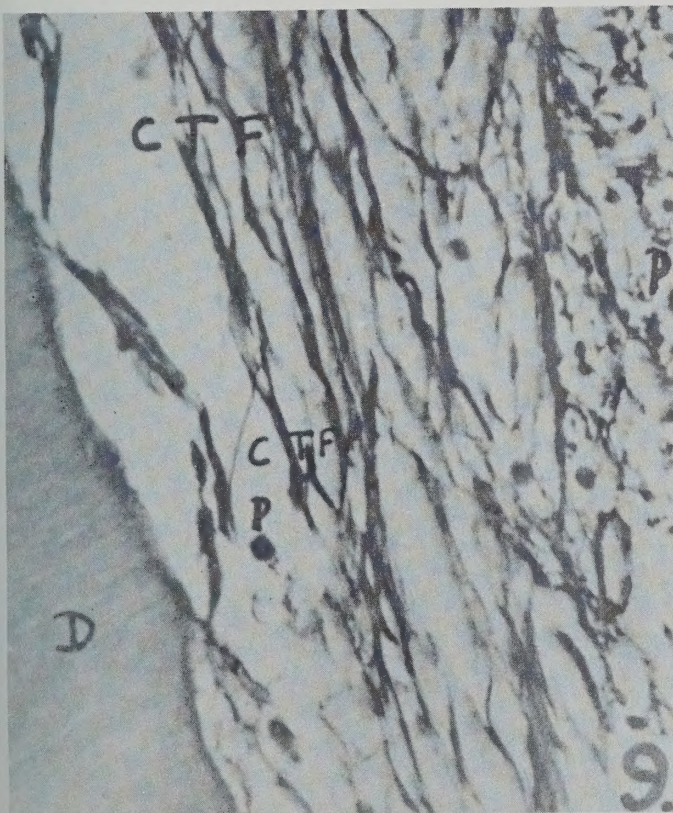
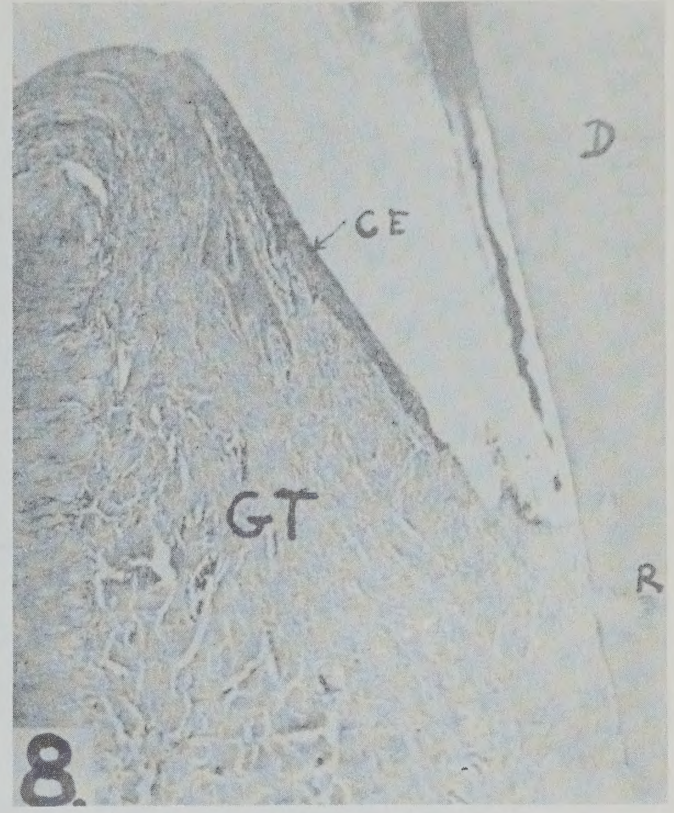
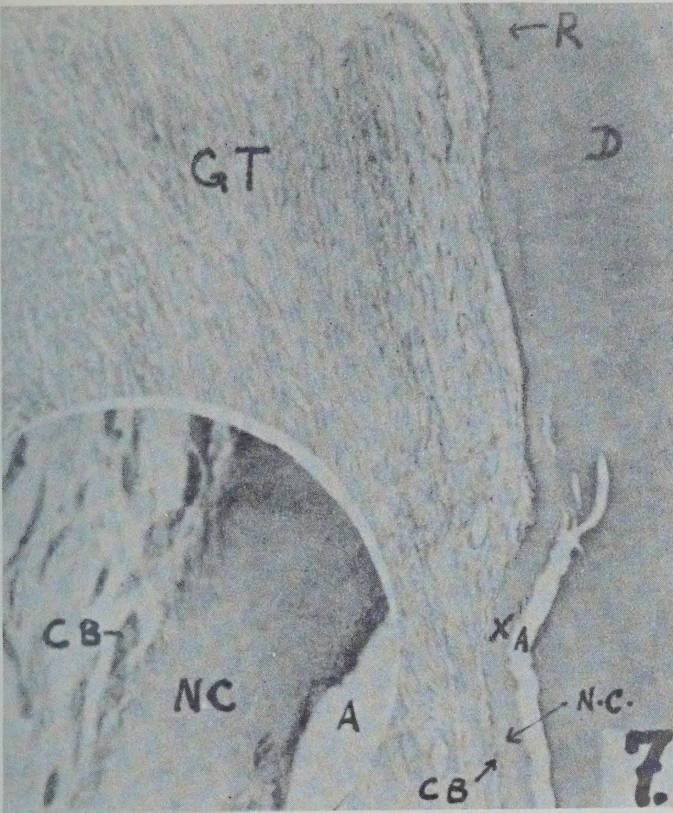








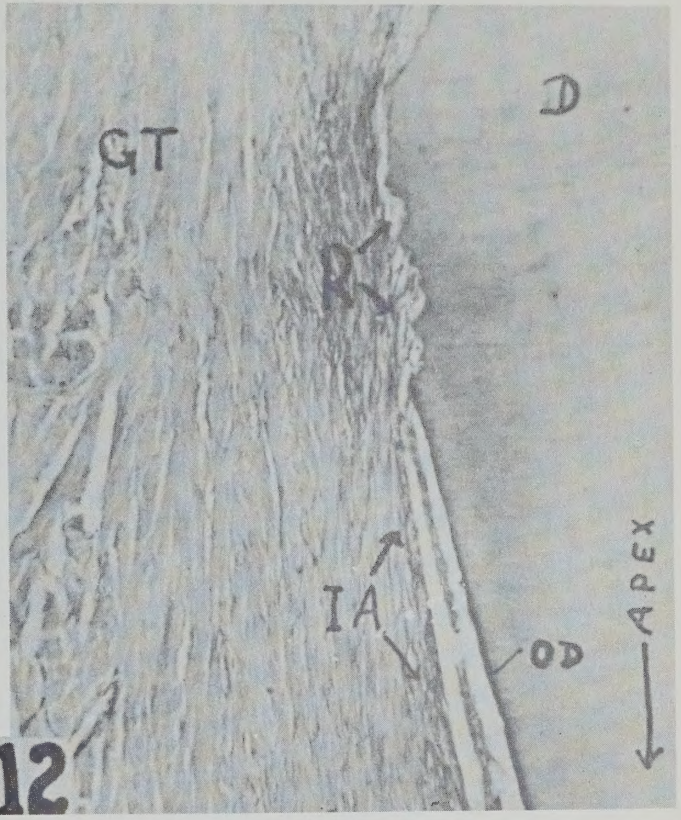
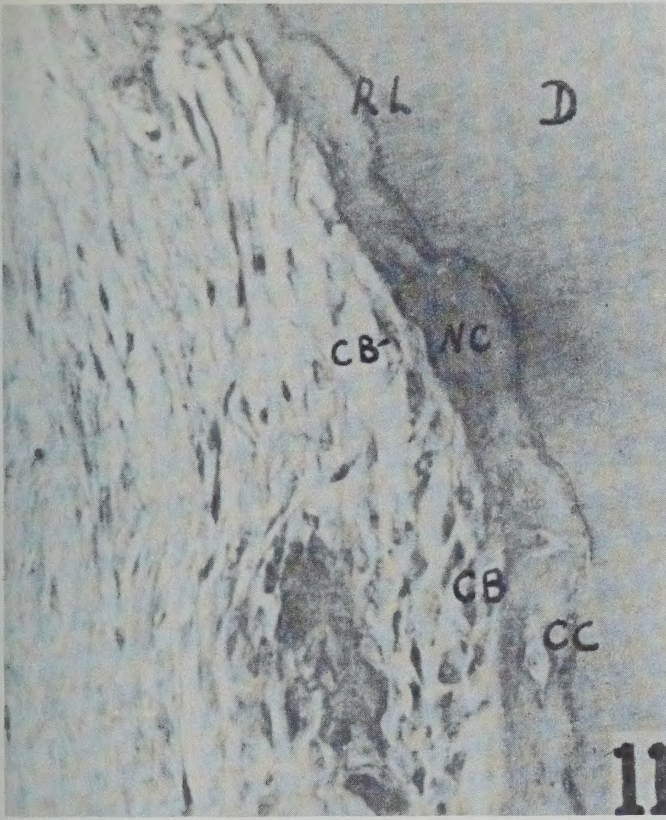




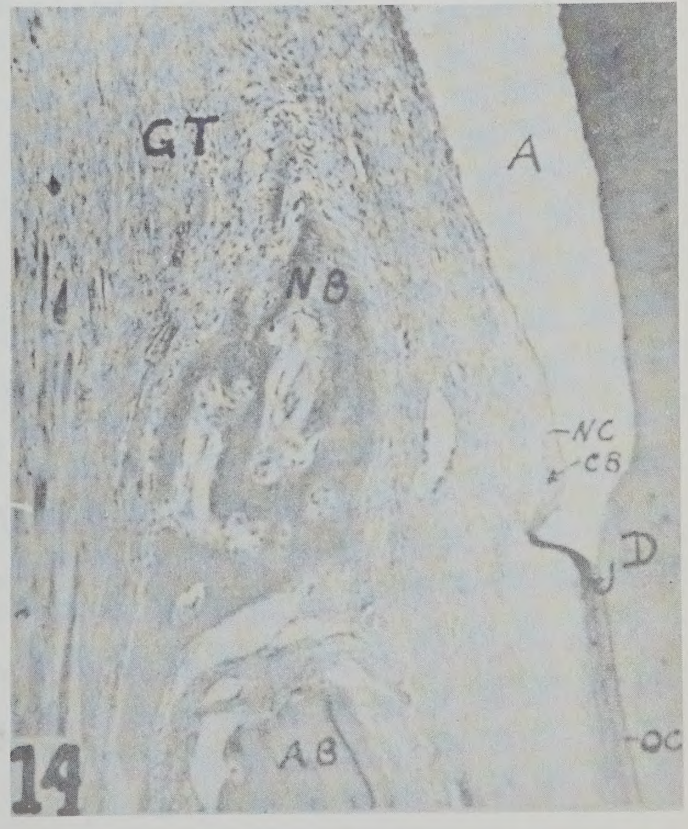
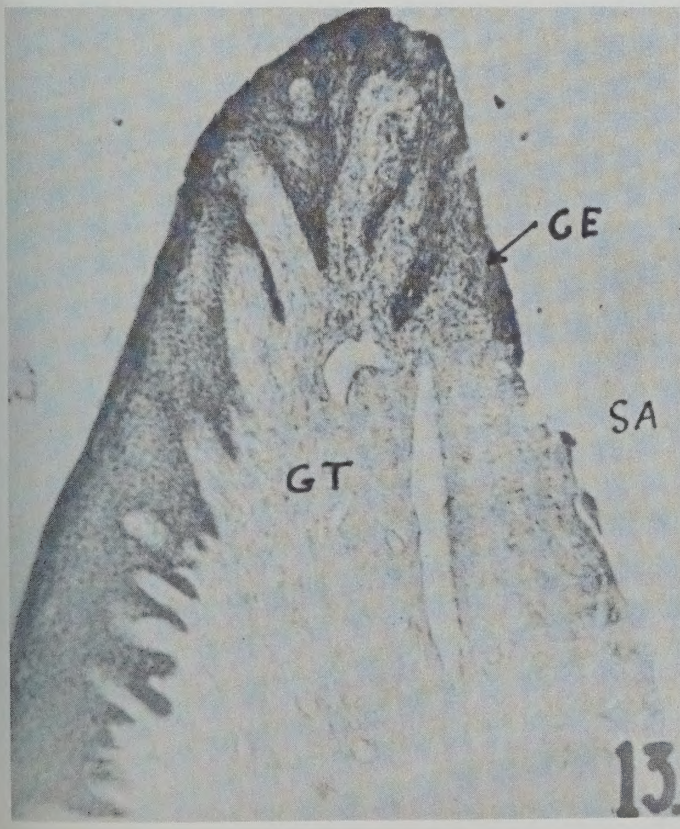








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## APPENDIX "G"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: Chemical Mechanisms in Dental Caries.  
Grantee: J.J. Rae (C.T. Clegg, J.T. Dentay, W.R.Kenzie)  
Project: DR 1

#### SUMMARY OF WORK

This annual report consists of 5 sub-sections.

##### A. Salivary Amylase and Dental Caries (Dentay)

There is no relationship between amylase activity of saliva and dental caries or between chloride concentration of saliva and dental caries.

##### B. Ammonia Production (Clegg).

As previously reported salivas on standing produce ammonia and contrary to Kesel's findings there seems to be no relation between ammonia production and dental caries. Certain amino acids seem to act as sources of ammonia.

##### C. Urea and Urease (Clegg)

There is a urease present in saliva but no urea. The urease in saliva and the ammonia producing mechanism are both removed by shaking with permutit and both are inhibited by glucose.

##### D. Organic Fluorides (Kenzie)

Propionyl fluoride has been prepared and its properties are being investigated with a view to its use in the topical application of fluorine to teeth.

##### E. Tooth Enamel and Lactobacillus (Clegg)

Powdered tooth enamel seems to inhibit the growth of l.b.a. whereas tricalcium phosphate does not.

The work on sections B,C,D,E is being continued.







APPENDIX "G"

Interim Report No. 9

ANNUAL REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH  
OF THE NATIONAL RESEARCH COUNCIL

February 1, 1950

Subject: Chemical mechanisms in dental caries.

Grantee: J. J. Rae

Assistants: C. T. Clegg, J.T. Dentay, W.R. Kenzie

Project: DR 1

Period Covered: February 1, 1949 - February 1, 1950

Sub-sections:

- (A) Salivary Amylase and Dental Caries (Dentay)
- (B) Ammonia Production (Clegg)
- (C) Urea and Urease in Saliva (Clegg)
- (D) Organic Fluorides (Kenzie)
- (E) Effect of Powdered Tooth Enamel on Growth of  
l.b.a. (Clegg).







(A) Salivary Amylase and Dental Caries

Amylase, one of the most common salivary enzymes, has been investigated since 1875. Nasse (1) (Arch. Ges. Physiol. (Pflügers). 9, 138, 1875) stated that salivary amylase "requires chloride" in order to have any activity at all. Rockwood (J.A.C.S. 41, 228, 1919) stated that chlorides increase the activity of salivary amylase. DeMarko (2) (Arch. Physiol. 37, 56, 1937) reached the conclusion that the "degree of amylolytic activity is a function of the cellular elements in saliva". He did not consider that by simply changing the chloride concentration with "the cellular elements" remaining the same in number, the activity can be changed. In 1929 Willstatter, Bamann and Rohdewald (Zeit. Phys. Chem. 186, 85, 1929) reported that amylase appears to be the only enzyme secreted by saliva. This was confirmed by us when centrifuged saliva lost none of its amylase activity even though all the "cellular elements" had been removed.

G.D. Marshall Day (Dental Cosmos 76, 683, 1934) reported that the amylolytic activity of the saliva was on the average 13% greater in a group of caries-immune persons than in a group of caries-susceptible persons. Tower and Crane (J.D.R. 23, 413, 1944) found the opposite to be true, i.e. that the saliva of caries active persons has greater amylolytic activity than caries-immune persons.

Rockwood (J.A.C.S. 41, 228, 1919) found a decrease in activity of salivary amylase on the addition of fluorides. Volker (Tufts Outlook 21, No. 2, 1947) agreed with the above statement, while McClure (U.S. Public Health Reports, 54, 2165, 1939) found that fluorides have an effect on the amylolytic activity of saliva.

EXPERIMENTAL

(a) Collection of Saliva

The saliva was collected from patients in the Dental Clinic of the Faculty of Dentistry, University of Toronto and from Cadets in training at No. 1 Air Training Station, Royal Canadian Air Force in Toronto. This latter group consisted of healthy young men from 18-26 years of age. The saliva was collected with stimulation and the condition of their teeth and gums was transcribed from their dental records.

(b) Determination of Amylase

To determine the amylase activity of the saliva a standardized Wohlgemuth procedure for  $\alpha$ -amylase was used. This consisted of a colorimetric comparison between samples of hydrolysis mixture blown into an iodine solution at 15 to 30 second intervals and a colorimetric standard made by adding 1 ml. of a



dextrin solution to an iodine solution. The colors were compared visually with reproducible results of sufficient accuracy. (Sandstedt R.M., Kneen and Blish. *Cer. Chem.* 16, 712, 1939).

In view of the fact that P. Bernfeld and Fuld (*Helv. Chim. Acta* 311, 1423, 1948) have shown that the ratio of saccharogenic activity to dextrinogenic activity for salivary amylase is unity we felt that it was unnecessary to determine the saccharogenic activity of the salivary amylase.

#### (c) Determination of Chloride

Chloride concentration of the salivas was determined by Shales' and Shales', (*J.B.C.* 140, 879, 1941) microvolumetric method. In this procedure a whole saliva sample is titrated with mercuric chloride in the presence of diphenyl carbozone until a permanent purple color is obtained. The accuracy of results was corroborated by means of the usual silver nitrate gravimetric procedure. The results are shown in Table I.

#### (d) Determination of Amylase Activity

The hydrolysis mixture consisted of 10 ml. of buffer pH 6.6 (potassium dihydrogen phosphate and sodium hydroxide), 10 ml. 2% soluble starch solution (Baker's Analyzed), 1 ml. of centrifuged diluted saliva (1:1). The mixture was immersed in a 37° bath and agitated during the experiment. The unit of amylase activity is defined as the amount of amylase required to hydrolyse 1 gram of starch in 1 hour at 37° to the specified red-brown end point. The results are shown in Table I.

### DISCUSSION

The amylase activity of saliva depends not only on the quantity of amylase present but also on the chloride concentration of the substrate. To make a reliable comparison of the amylase activity of salivas the number of variables should be reduced. This would involve comparing the amylase activity of salivas at the same chloride concentration which could be achieved by removal of most of the chloride by dialysis before determining amylase or by determining the chloride content and adjusting them all to a figure represented by the highest value. Either of these procedures involves difficulty. Dialysis always results in loss of amylase activity and in the second case the values for chloride concentration show such a wide variation that to choose a postulated maximum is difficult.

Since we are most interested in the possible relationship between amylase activity and dental caries it should be pointed out that amylase activity consists of two parts;- amylase content and the activating effect of chloride. The



"G-3"

TABLE I

Amylase Activity, Chloride Concentration and Dental Caries

| NO. | Cavities<br>and filling | Amylase<br>Activity | mg. %<br>Cl |
|-----|-------------------------|---------------------|-------------|
| 1   | 0                       | 18.0                | 84.0        |
| 2   | 0                       | 10.7                | 58.8        |
| 3   | 1                       | 22.0                | 77.6        |
| 4   | 2                       | 4.9                 | 51.1        |
| 5   | 2                       | 41.0                | 78.0        |
| 6   | 2                       | 12.0                | 78.0        |
| 7   | 2                       | 3.8                 | 68.4        |
| 8   | 3                       | 18.0                | 62.4        |
| 9   | 3                       | 20.6                | 64.4        |
| 10  | 3                       | 14.4                | 58.1        |
| 11  | 4                       | 14.4                | 76.1        |
| 12  | 4                       | 24.8                | 65.8        |
| 13  | 4                       | 24.0                | 44.4        |
| 14  | 4                       | 6.0                 | 57.8        |
| 15  | 5                       | 24.0                | 74.4        |
| 16  | 5                       | 6.8                 | 77.4        |
| 17  | 5                       | 41.0                | 56.4        |
| 18  | 6                       | 4.1                 | 75.6        |
| 19  | 6                       | 1.2                 | 72.0        |
| 20  | 7                       | 13.7                | 87.7        |
| 21  | 7                       | 13.1                | 102.5       |
| 22  | 7                       | 22.0                | 52.1        |
| 23  | 8                       | 22.0                | 76.1        |
| 24  | 8                       | 1.1                 | 57.8        |
| 25  | 8                       | 32.0                | 69.6        |
| 26  | 8                       | 12.0                | 88.1        |
| 27  | 9                       | 22.0                | 68.4        |
| 28  | 12                      | 24.0                | 79.4        |
| 29  | 14                      | 32.0                | 74.4        |
| 30  | 16                      | 35.0                | 79.6        |
| 31  | 20                      | 16.0                | 76.8        |



relationship between amylase content and dental caries cannot be determined without isolation and determination of the dry weight of the crystalline amylase. This procedure is involved and time consuming. Unless experiments are performed in which the chlorides of salivas are all adjusted to the same level or a determination is made of dry weight amylase content we can only refer to the "amylase activity" of saliva.

### SUMMARY

Confusion exists with respect to the amylase activity of saliva and its possible relationship to dental caries. Part of this confusion arises from the fact that chloride ion is an activator for amylase and no satisfactory method has been developed for determining amylase content (dry weight). The results show no relationship between amylase activity and dental caries or between chloride concentration and dental caries.

#### (B) Ammonia Production

When saliva is allowed to stand for 24 hours a considerable amount of ammonia is produced.

Kesel (J.A.D.A. 33, 695, 1946) reported that low l.b.a. count saliva produced ammonia on 8-day incubation whereas high l.b.a. count saliva produced little or no ammonia under the same conditions.

In our last report (No. 7) we showed that a period of 24 hours is sufficient to measure ammonia production; that the optimum pH for its production is 8.5; that the ammonia producing mechanism (A.P.M.) is removed by shaking the saliva with permittit; that the A.P.M. is inhibited by boiling and by the addition of antiseptics (merthiolate) and glucose (2%). We were unable to confirm Kesel's results and found that no marked difference in the A.P.M. production between high and low count l.b.a. salivas existed. In all cases considerable amounts of ammonia were formed.

Possible sources of ammonia production from saliva are by the action of enzymes on the various nitrogen containing compounds in saliva.

One such mechanism is the production of ammonia from urea by urease and it has been shown in Section C that a urease is present in saliva. Unfortunately, no urea has been found by us in salivas.

Another possible source of ammonia is the amino acids present in saliva. It has been shown that the A.P.M. of saliva is capable of producing ammonia from solutions of



"G-5"

serine (386) asparagine (356, 383) and d-glutamic acid (381). It has also been shown that cystine (383) and aspartic acid (356, 381) under the same conditions did not produce any ammonia by the action of saliva.

The proteins, casein, gelatin and egg-albumin, when added to saliva which was subsequently incubated, did not cause any increase in ammonia production.

(c) Urea and Urease

As mentioned in our last report, since urease is an ammonia-producing enzyme, its presence in saliva might account for at least part of the A.P.M. Experiments have shown that there is no urea in saliva (331, 333, 334). The presence of urease was demonstrated and the results are shown below (333, 334).

|                                            | A<br>mg. % $\text{NH}_3$ | B    |
|--------------------------------------------|--------------------------|------|
| Saliva + urea after 24 hours incubation    | 129                      | 219X |
| Saliva (no urea added)                     | 25.8                     | 23.8 |
| Saliva + urea after 1 1/2 hours incubation | 28.4                     | 19.3 |
| Saliva (no urea added)                     | 15.0                     | 6.4  |

It has been demonstrated that urease in saliva and the A.P.M. of saliva are alike in the following particulars:

(a) They are both removed by shaking with permutit (353)

(b) They are both inhibited by the addition of glucose.

The urease in Jack Bean Meal behaves in a similar manner, i.e. it is also removed by permutit (353) and inhibited by glucose (354).

It is intended to investigate the relationship between l.b.a. count and urease content of a series of salivas.



(D) Organic Fluorides

In view of the fact that the topical application of fluorides seems to exert a beneficial effect on dental caries it was thought that some less toxic fluorides might be used for this purpose and we turned over attention to organic fluorides.

One of the simplest less-toxic organic fluoride is propionyl fluoride ( $C_2H_5COF$ ) and a literature survey of its preparation and properties was undertaken.

There are two likely looking methods for its preparation: (i) a method proposed by Meslans (Bull. Soc. Chim. 3, 15, 877) which involves the reaction between propionyl chloride and zinc fluoride; (ii) a method proposed by Mashenstsev (J. Gen. Chem., U.S.S.R. 16, 203, 1946) which uses as starting materials benzoyl fluoride and propionic acid.

Since such out-of-the-way chemicals as zinc fluoride, propionyl chloride and benzoyl fluoride cannot be purchased readily they have to be made and this was done.

The fluorides cannot, of course, be kept in glass apparatus so it was necessary to devise apparatus made from copper or aluminum or use a suitable protective coating on the glass apparatus used.

Several runs have been made on the preparation of propionyl fluoride by Meslan's method with varying success.

The properties of the product such as effect on container, hydrolysis, stability are now being studied.

As a by-product of this research a few improved syntheses using propionyl fluoride as a starting material are to be investigated.

(E) Effect of Tooth Enamel on Tricalcium Phosphate on The Growth of Oral Bacteria (l.b.a.)

In describing a method for determining the number of micro-organisms in rat teeth, Wakeman, Smith et al (J.D.R. 27, 41, 1948) found that ground teeth gave a count ten times greater than when whole teeth were used. They concluded from this that the increase observed was due to the micro-organisms contained in the pits and fissures. However, it may also be concluded that ground tooth substance is a good culture media addendum for such micro-organisms. To test this assumption the following experiments were performed:

1 ml. saliva mixed with 4 ml. beef broth was shaken at 20° for 20 hours and 0.1 cc. of the culture plated and incubated



"G-7"

for 4 days at 37°. The l.b.a. count was then determined. Under the same conditions the addition of 50 mg. of 10 mesh ground tooth enamel (Manly and Hodge) inhibited almost completely the growth of l.b.a.

The counts obtained after the above treatment when no tooth enamel was added were comparable to those obtained on the original saliva (380).

In another experiment to see if this rather interesting inhibitory effect of powdered tooth enamel on the growth of l.b.a. would be also shown by tricalcium phosphate the results were inconclusive but again inhibition by the powdered tooth enamel was observed. This work is being continued.







APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Subject: The relining of March, 1950  
Grantee: Dr. A. E. R. Westman  
Subject: Cements for methacrylate inlays  
Grantee: Dr. A. E. R. Westman  
Project: DR 12

SUMMARY OF WORK

Work on this project has been held in abeyance pending the developments on the closely-related project DR 25 on direct acrylic fillings.

Although important advances have been made recently in the commercial field of direct acrylic inlays, there are indications that in some cases the use of indirect acrylic inlays may be essential. In view of this situation it is recommended that this project be kept open pending the further experimental clarification of the properties of acrylic fillings.

Date: January 27, 1950.

"O. J. Schierholtz"  
Signature

for

A.E.R. Westman.







APPENDIX "I"  
REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: The relining of lower dentures  
Grantee: Dr. A. E. R. Westman  
Project: DR 21

SUMMARY OF WORK

This project originally began as an investigation of the means of preventing distortion of dentures during repairs. In the course of the work, it was found that there were, in fact, two problems. One of these, the repair of fractures and the replacement of teeth, has been dealt with successfully and the findings published recently in the Journal of the Canadian Dental Association. The second problem concerned the prevention of distortion in lower dentures on relining. This problem was considered important enough to warrant immediate attention and the continuation of this project for a period longer than was originally anticipated.

At the present time, we have completed experimental studies on the relining of lower dentures, which indicate that a successful relining method can be realized. There remain to be done on this project some practical trials and possibly a chemical investigation of the mechanism. It is intended that these further studies will be completed by the end of the term of this project, that is, March 31, 1950. At that time a final report will be submitted.

Date: January 27, 1950.

"O.J. Schierholtz"  
Signature







## APPENDIX "I"

### ONTARIO RESEARCH FOUNDATION

Department of Chemistry

Dental Materials Research Fellowship

Project DR 21

Report 5001

#### Scope of This Report

This report summarizes the work which has been done to date on the problem of relining lower dentures. It describes a technique which has been successful for preventing distortion of experimental dentures.

#### Distortion on Relining

A summary report on Project DR 21 was submitted to the Associate Committee on Dental Research a year ago. That report, however, covered only the prevention of distortion in dentures undergoing repairs for mechanical failures such as fractures and loss of teeth. The equally important problem of preventing distortion in lower dentures on relining had not been solved satisfactorily at that time. On the basis of laboratory experiments, it now appears that a suitable solution can be given.

#### Cause of Distortion on Relining

The factors which cause distortion on relining were clearly shown by experiments with flat strips of denture-base material measuring approximately  $1/8 \times 1 \times 3$  inches. The addition of a thin layer of new denture-base material caused bowing of the whole strip so that the reference or untouched surface became convex in shape. This result occurred no matter whether the reline was carried out at room temperature or at  $212^{\circ}$  F. and indicates that the distortion is caused by stresses set up in the new material as it contracts on polymerization. The same effect is evident in relined lower dentures by the fact that the heels are "pinched in" so that the dentures will no longer fit their casts or occlusal indices.

#### The Elimination of Reline Distortion

Two methods have been suggested for overcoming reline distortion. Both methods have as their objective the production of a flexible reline resin which will not warp the original material.



The first method makes use of a permanently flexible resin in the relining material. Several of the higher alkyl methacrylates such as ethyl, butyl, octyl and lauryl methacrylates have been investigated to some extent. The monomers of these compounds, with the exception of ethyl methacrylate, are, however, poor solvents for the methyl methacrylate polymer ordinarily used and are not suitable for use in the usual manner of making a dough with monomer and polymer. Moreover, ethyl and butyl methacrylates do not give resins with enough flexibility for the purpose. Octyl and lauryl polymethacrylates, used as 10-20% solutions in methyl methacrylate monomer, and used as the liquid component, can be employed successfully with methyl polymethacrylate powder for making relines without distortion. However, the necessity of polymerizing these methacrylates before making the solutions in methyl methacrylate, the tacky nature of the solutions and to a slight extent their yellow color, are factors which make the second method to be described preferable.

The second method makes use of a polymerization inhibitor added to the monomer. The inhibitor would tend to cause the formation of a low-molecular-weight polymer with some degree of flexibility.

Hydroquinone was first investigated but was found to be effective in preventing distortion only for a period of from ten days to two weeks after relining. After this time, relines began to warp and become progressively worse. A yellow or orange discoloration in the relining material suggested that the hydroquinone was being oxidized and therefore losing its inhibitory effect.

A more stable compound was tried and found to be suitable. It was 2,5-ditertiary butyl hydroquinone. Flat strips and dentures were relined with this material in the monomer, along with trihexylamine, and no distortion has been observed. Some of these strips and dentures have been conditioning at room and body temperatures for periods of two to four months and appear to be dimensionally stable.

The relining monomer consists of hydroquinone-free methyl methacrylate, 0.05%, 2,5-ditertiary butyl hydroquinone and 3% trihexylamine (a polymerization accelerator). Just before adding dental grade methyl methacrylate polymer to make the mixture, 0.25% benzoyl peroxide is added to the monomer and dissolved. Curing is carried out at 135° F. (to avoid thermal distortion of the denture) for a period of 3½ hours.

Although the relined area is relatively soft (it has a Knoop hardness number of about 11, compared with fully-cured polymer of 16 to 17), it is not apt to be scratched or otherwise damaged in its position next to the mouth tissues.



Future Work

It is considered advisable to carry out a few clinical tests with this method in order to ensure that our laboratory experiments can be adapted to practical work. It has been suggested that the incompletely-cured reline material may be a source of irritation to mouth tissues. This will have to be determined in actual trials.

It is intended that the research on this subject will be concluded at the expiration of the term of this project. A full report on relining will then be submitted.

January 31, 1950

(SIGNED:) Douglas R. Christie

Douglas R. Christie  
Research Fellow







APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: Direct acrylic fillings

Grantee: Dr. A.E.R. Westman

Project: DR 25

SUMMARY OF WORK

Before the experimental program was started on this project it was thought wise to determine the effect of the materials and techniques used on the pulp of vital teeth, since there appeared to be no record of this type of work in the literature.

Consequently, the research on this project has dealt with a study of the above-mentioned effects using dogs as experimental animals.

Since considerable work is involved in the preparation and sectioning of the large number of teeth required to place the experiment on a statistically-sound basis, this phase has been somewhat prolonged. The histopathological study of the teeth is now above one-half completed. The results are encouraging but no definite statement can be made about the final result at this time.

A critical examination is also being made of the suggested methods for evaluating acrylic filling from a practical standpoint in order to have a reliable criterion of the quality of various acrylic preparations. At the same time we are proceeding toward the preparation of acrylic resins of varying composition to obtain the required physical properties.

Progress reports will be submitted, as is our custom, at more or less regular intervals depending on the status of the research.

Date: January 27, 1950.

"O. J. Schierholtz"







APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: The effects of hard and soft diets on the supporting structures of the teeth

Grantee: Dr. C.H.M. Williams

Project: DR 24

SUMMARY OF WORK

Since last reporting to the Committee in September, 1949, serial sections of the lower first molar tooth and its supporting structures have been prepared and studied. The gingival tissue has been studied in detail and also the periodontal membrane and alveolar bone.

Changes in the gingival tissues have been observed associated with wear on the occlusal surface of the teeth and a movement of the teeth towards the occlusal plane. It is also considered that pathological changes have taken place in the soft diet group, possibly associated with the contents of the gingival crevice.

These observations have been made on four month old rats and it is considered that much additional information will be gained from a study of adult animals and from animals that are being fed experimentally for a longer period of time. Further material is being studied and if similar differences are found, then it is felt that these changes are a matter of some significance in periodontal pathology.

(Signed;) Chas. H.M. Williams  
grantee







## APPENDIX "K"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: The effects of hard and soft diets on the supporting structures of the teeth

Grantee: Dr. C.H.M. Williams

Project: DR 24

This study was commenced in September, 1948 and the macroscopic observations on the growth and development of the jaws were presented to the committee in September, 1949. This report is based on a microscopic study of serial sections cut in a bucco-lingual plane through the lower left first molar tooth. Four blocks of tissue taken from the hard diet specimens and six blocks from the soft diet specimens have been studied. The tissue was embedded in celloidin, stained in hematoxylin and eosin, and the following observations made.

#### 1. GINGIVAL TISSUE:-

This consists of three components:-

- (a) Oral epithelium, which proliferates downwards for a varying distance behind the epithelial attachment.
- (b) Epithelial attachment, which is that epithelium normally in organic connection with the tooth surface.
- (c) Lamina propria or subepithelial connective tissue.

#### (a) Oral Epithelium:-

In the hard diet specimens the epithelium was covered with a thick, well-stained layer of keratin. The most prominent epithelial layer was the stratum granulosum, which consisted of many layers of elongated cells with darkly stained nuclei that were tightly packed and orderly arranged. The stratum spinosum was confined to one or two layers in thickness.

In the soft diet specimens, the keratin layer was thinner than in the hard diet specimens and in some cases almost absent. The bulk of the epithelium consisted of the stratum spinosum which was composed of many layers of pale, polyhedral cells. The stratum granulosum was of varying thickness, but the cells were not as closely packed together nor their nuclei as darkly stained.



The epithelium which had proliferated downwards behind the epithelial attachment presented similar characteristics. It was keratinized, much more so in the hard diet specimens than in the soft diet specimens, and in some of the soft diet tissue the epithelium appeared to be in an early stage of degeneration,—the nuclei were pale and the cytoplasm vacuolated. In the soft diet specimens, the flattened, darkly staining cells of the stratum granulosum were inconspicuous. This epithelium in the hard diet tissue had proliferated down behind the epithelial attachment to the cemento-enamel junction and sometimes below it, while in the soft diet tissue, it had only extended a short distance behind the epithelial attachment so that it was found well above the cemento-enamel junction.

(b) Epithelial Attachment:—

In the hard diet specimens the attachment was not found to be as high up on the enamel surface as in the soft diet specimens. In the hard diet tissue there was a moderate degree of epithelial downgrowth along the cementum, both on the buccal and lingual aspects of the tooth. The epithelium was thick, the cells squamous with darkly staining nuclei lying parallel to the enamel surface. Slight neutrophilic infiltration was evident.

In the soft diet specimens the attachment remained high up on the enamel surface and there was no epithelial downgrowth along the cementum on the buccal aspect, except in one case, but there was slight downgrowth on the lingual aspect. The epithelium was thin, the nuclei ovoid and palely stained, and there was a moderate to marked neutrophilic and lymphocytic infiltration. In some specimens these cells and some of the epithelial cells appeared pyknotic. The epithelium presented various stages of degeneration and in at least one specimen, the tissue was considered necrotic.

In the majority of the hard diet specimens examined there was no gingival crevice. However, in most of the soft diet specimens a shallow crevice filled with debris was observed.

(c) Lamina Propria:—

In the hard diet tissue the lamina propria consisted of dense irregularly arranged connective tissue, which was not highly vascularized. A few neutrophils and lymphocytes were seen in relation to the epithelial attachment on the cementum.

In the soft diet tissue a different picture was observed and the condition of the connective tissue varied with that of the epithelial attachment. When the epithelial



attachment appeared intact and the degenerative changes were not of a marked degree, there was a moderate degree of neutrophilic and lymphocytic infiltration and the capillaries appeared somewhat dilated. But when the attachment was in a more advanced degenerative condition, a great number of lymphocytes were seen in the lamina propria adjacent to the epithelial attachment, a large proportion of which were pyknotic. Very few neutrophils were observed associated with this condition and the tissue was not highly vascularized. Another feature of this condition was a change in the intercellular substances in the region of the base of the epithelial attachment. Here the collagen fibres appeared disorganized and they stained poorly. In some specimens there was a certain degree of hemorrhage at the base of the epithelial attachment.

## 2. PERIODONTAL MEMBRANE AND ALVEOLAR BONE:--

No obvious changes were observed in these structures between the hard diet and the soft diet specimens. However, when additional material becomes available a further study will be made.

### Discussion:--

While it is realized that no definite conclusions can be made from such a limited amount of material, it is thought that three main changes have taken place:--

- (a) Variations in the oral epithelium associated with a difference in the degree of functional stimulation.
- (b) A change in the position of the epithelial attachment on the tooth surface as a result of wear on the occlusal surface and a movement of the tooth towards the occlusal plane.
- (c) Pathological changes in the epithelial attachment and the lamina propria of the soft diet group, which are possibly associated with the contents of the gingival crevice.

These observations have been made on four months old rats and it is considered that much additional information could be gained from a study of adult animals and from animals that have been fed experimentally for a longer period of time. Further material will be studied and if similar differences are found, then it is felt that these changes are a matter of some significance in periodontal pathology.



The study for the next year has been planned as follows:-

Three groups of animals (forty rats in each group) are at present being fed hard and soft forms of the same diet. One group of adult male rats, averaging 435 grams at the beginning of the experiment have been divided into two equal groups and one group is being fed the hard food and the other the soft food. These animals were reared on hard food up to the time of experimentation. They will be sacrificed at the beginning of March, 1950 and their mandibles will be examined macroscopically and microscopically.

The second group consists of forty weanling male rats which are being reared on a soft diet until their body weight reaches approximately 400 grams. They will then be divided into two equal groups and one group will be given hard food while the other group will be given soft food for a four month experimental period.

The third group consists of male and female rats that have been divided into two equal groups, one group being fed hard food and the other soft food from the time of weaning. These animals will not be sacrificed until they are a year old (November, 1950).

When this material has been completely examined, and this should be accomplished within the coming year, it is thought that the study on experimental animals will be completed. It is then hoped that a study on human beings will be undertaken. This could be accomplished with the co-operation of an orphanage or similar group of children, to observe the effects of the physical consistency of food on the growth of the jaws and on the health of the supporting structures of the teeth.

Chas. H.M. Williams  
grantee



## APPENDIX <sup>ML</sup> 00

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: Improvement of Sub-gingival curettage performed in the treatment of the periodontal pockets

Grantee: Dr. Jules Thébaud

Project: DR 20 Amount of Grant: \$750.

#### SUMMARY OF WORK

##### 1 - CURETTING OF THE CEMENTAL WALL -

Scaling and finishing of 61 teeth were performed in the following order:

| PROCEDURE    | INSTRUMENTATION              |                          |
|--------------|------------------------------|--------------------------|
|              | on another side of the roots |                          |
| A) Scaling   | 1)                           | Hoe (only)               |
|              | 2)                           | Hoe overused             |
|              | 3)                           | Hoe used without fulcrum |
|              | 4)                           | Hoe and curette          |
|              | 5)                           | Curette (only)           |
|              | 6)                           | Curette overused         |
|              | 7)                           | Curette                  |
|              | 8)                           | Hoe and curette          |
| B) Finishing | 9)                           | File (only)              |
|              | 10)                          | Carborundum disk         |
|              | 11)                          | Abrasive steel disk      |

Most of the effects produced during the scaling and the finishing processes were microphotographed.

It was found that the two processes can be combined and improved in performing the scaling first with the hoes applied with proper fulcrum stand, then sharp curettes with routine planing motions followed by the use of dull curettes which brought up a burnished and finished surface. The thinning of the cement by over-use of the hoes and the curettes was also recorded by microphotographs.



(SUMMARY)

The use of the cemental file was proved to be harmful to the cement, and liable to create an irregular and grooved surface which is not suitable for tissue repair or readaptation.

## 2 - CURETTMENT OF THE BASE (OR BOTTOM) OF THE PERIODONTAL POCKETS

The outline of a technique for an improved and a more accurate curettage of the bottom of the pockets is also described; considering thus, this phase of the sub-gingival curettage as a special step in the operation, the third one to be used after the curettment of (1st) the cemental wall and of (2nd) the soft side wall. Histological proofs for checking the accuracy of that method shall be developed later on in using biopsy materials.



## APPENDIX "L"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: Improvement of sub-gingival curettage performed in the treatment of the periodontal pockets.

Grantee: Dr. Jules Thébaud

Project: DR 20

An average of 122 hours was spent to carry out our final experiments on the cemental wall of the periodontal pockets. As credit was renewed only in June 1949, the investigations were performed at the beginning of the academic year, in September last, until the middle of December.

To complete our first series of 36 specimens, 10 more slides, were prepared, and examined together with a second series of 25 teeth which were treated "in situ" while being involved with pathosis. Among those specimens, 15 were decalcified and 10 were prepared in ground sections.

#### CURETTEMENT OF THE CEMENTAL WALL OF THE PERIODONTAL POCKETS -

The curettement of the cemental surface being the first phase to be performed in the sub-gingival curettage method, we believe that this procedure so currently undertaken by the students as well as by the practitioners, should be microscopically recorded and analyzed, taking in consideration, at the same time, the various histologic features brought up on the surface of that tissue. Some authors have expressed their opinions as to the efficiency of the routine planing curettage combined with the spot curettage, others have stressed the inadequacy of curetting the cement with the current scalers which are considered too large for the removal of all the minute deposits.

However, no previous study has been published yet in the dental literature in regard to details in this type of procedure, so as to record:

- a) the effect produced by the various instruments when used separately during the curettage.
- b) the combined effect of those instruments used in a conservative manner.
- c) the gouging of the cemental substance and the liability of its thinning at the gingival half when the instruments are overused or wrongly applied.







TECHNIQUE -

Scaling and finishing were performed on teeth on which there was a certain amount of calcareous deposits, both supra and infra-gingival. These specimens were mounted on plaster blocks for the operation of scaling. The teeth involved with pathosis and surrounded by pockets varying from 4 to 7 millimetres were treated "in situ".

For scaling we have used the ordinary hoes Nos. 3,9,7S and 9S and the McCall's curettes Nos. 13,14,17 and 18 with the routine planing motions.

In the finishing process we have used the ordinary cemental files, and with hand pressure some abrasive materials, such as, portions of carborundum disks and fine metal abrasive disks, in order to record the accuracy and the difference between those materials. We have also recorded the combined effects of the scaling and the finishing processes on the cement surface.

Instrumentation was done in the following order:

| PROCEDURE:               | Instrumentation<br>on one side of the roots |                                   | Instrumentation<br>on another side of the roots |  |
|--------------------------|---------------------------------------------|-----------------------------------|-------------------------------------------------|--|
|                          |                                             |                                   |                                                 |  |
| A) Scaling               | 1)                                          | none (untreated cemental surface) | Hoe (only)                                      |  |
|                          | 2)                                          | none " "                          | Hoe overused                                    |  |
|                          | 3)                                          | none " "                          | Hoe used without fulcrum                        |  |
|                          | 4)                                          | none " "                          | Hoe and curette                                 |  |
|                          | 5)                                          | none " "                          | Curette (only)                                  |  |
|                          | 6)                                          | none " "                          | Curette overused                                |  |
|                          | 7)                                          | hoe                               | Curette                                         |  |
|                          | 8)                                          | hoe                               | Hoe and curette                                 |  |
| B) Finishing             | 9)                                          | none (untreated cement)           | File (only)                                     |  |
|                          | 10)                                         | none " "                          | Carborundum disk                                |  |
|                          | 11)                                         | none " "                          | Abrasive steel disk                             |  |
| C) Scaling and Finishing | 14)                                         | hoe                               | Hoe and file                                    |  |
|                          | 15)                                         | curette                           | Curette and file                                |  |
|                          | 16)                                         | curette                           | Curette and abrasive steel                      |  |
|                          | 17)                                         | hoe and curette                   | Curette, hoe and carborundum                    |  |
|                          |                                             |                                   |                                                 |  |
|                          |                                             |                                   |                                                 |  |

In addition to table A included in our report No. 3, March 1949, the following data were recorded after the examination of the other specimens.







TABLE B

| SERIAL NO. | PATIENT | TEETH                 | POCKETS |     |         | SIDE TREATED | INSTRUMENTATION                          |                             |
|------------|---------|-----------------------|---------|-----|---------|--------------|------------------------------------------|-----------------------------|
|            |         |                       | 4       | m/n | distal  |              | Performed on one side only               | Effect of scaling           |
| A          | H.J.    | 2nd upper lt.bicuspid | 4       | m/n | distal  |              | file                                     | Irreg.and thinned           |
| B          | E.B.    | 2nd lower lt.molar    | 5       | m/n | distal  |              | hoe and curette                          | Reg. and slightly thinned   |
| C          | H. J.   | 2nd upper rt.molar    | 4       | m/n | distal  |              | curette                                  | Normal thickness and reg.   |
| D          | R.L.    | 1st rt.upper molar    | 5       | m/n | palatal |              | hoe                                      | Irreg.outline               |
| E          | R.L.    | 2nd rt.upper molar    | 4       | m/n | mesial  |              | hoe and curette                          | Reg.and thinned             |
| F          | H.L.    | rt.upper cuspid       | 7       | m/n | distal  |              | hoe,curette and file                     | Irreg.outline               |
| G          | R.L.    | 2nd lt.upper molar    | 6       | m/n | distal  |              | curette and file                         | Irreg.outline               |
| H          | H.L.    | 3rd rt.upper molar    | 5       | m/n | distal  |              | curette and Irreg.and grooved abra.steel |                             |
| I          | E.A.    | 2nd upper lt.molar    | 5       | m/n | mesial  |              | hoe and curette                          | Clear outline               |
| J          | E.A.    | 2nd upper rt.bicuspid | 6       | m/n | palatal |              | hoe and curette                          | Clear contour               |
| K          | E.A.    | lt.upper central      | 4       | m/n | distal  |              | hoe                                      | Slightly irreg.             |
| L          | L.G.    | 1st lt.upper molar    | 6       | m/n | distal  |              | hoe and curette                          | Clear outline               |
| M          | L.G.    | 2nd rt.lower molar    | 5       | m/n | distal  |              | curette and blunt curette                | Clear outline               |
| N          | P.E.B.  | 1st upper lt.molar    | 4       | m/n | distal  |              | curette(over-used)                       | Slightly grooved            |
| O          | P.E.B.  | 2nd upper lt.molar    | 5       | m/n | distal  |              | hoe(overused)                            | Gouged and exposed dentine. |







"L-4"

TABLE C

GROUND SECTIONS

| SERIAL<br>NO. | TEETH          | INSTRUMENTATION                                |                                        |
|---------------|----------------|------------------------------------------------|----------------------------------------|
|               |                | Only performed on buccal<br>side of the roots. | Effect of scaling.                     |
| 100           | upper cuspid   | curette                                        | Normal thickness                       |
| 101           | upper lateral  | hoe                                            | Uneven contour                         |
| 102           | upper central  | hoe and curette                                | Slightly thinned                       |
| 103           | lower cuspid   | file                                           | Very irregular contour                 |
| 104           | lower cuspid   | abrasive steel                                 | Slightly irregular<br>contour          |
| 105           | upper cuspid   | carborundum disk                               | Very irregular contour                 |
| 106           | upper cuspid   | hoe and curette                                | Normal thickness and<br>smooth outline |
| 107           | upper central  | curette and file                               | Thinned and uneven                     |
| 108           | lower bicuspid | curette and abrasive steel                     | Thinned and uneven                     |
| 109           | upper bicuspid | curette and carborundum<br>disk                | Thinned and uneven                     |







CONCLUSION

C1. THE SCALING -

Beside large and continuous masses of calcareous deposits over the roots, there are very many minute particles in which are imbedded some debris of the cement cuticula and adhesions of the detached fibres of the periodontal membrane. Fig.1 and 2.

This common condition in deep pockets explained the inaccuracy of the spot curettage technique during which the fine concretions may remain untouched (Fig. A); while the routine planing in which the curettement is performed in various directions makes it more easy to detect and remove all periodontal debris and concretions (Fig. B).

THE HOE -

In scaling most of the deep pockets, the moderate use of the hoe is the first step to be recommended, as the exposed surface of the root can be slightly cleaved and readily refreshed in detaching the larger calculi; but the hoe has a tendency, even when it is properly applied, to leave an uneven surface (Fig. 3) with very fine eminences, which should be scaled anew and finished with a curette, (Fig. 4).

Exposure of the dentine occurs when the scaling with the hoe is purposely overdone without taking any precaution to establish a correct fulcrum stand (Fig. 5). This created condition is not an obstacle to readaptation or complete healing of the pocket. It is true that the thinning of the cement is somewhat easily obtained with the overuse of that instrument (Fig. 6), as pointed out by Box.

C2. CURETTE -

As is commonly believed, the curette is really the ideal instrument for root curettement, specially when the most tenacious particles of calculus are previously removed with the hoe (Fig. 7,8,11 and 12). When curetting is overdone with a very sharp curette there is a slight tendency of thinning and breaking the regular and smooth outline of the cemental substance (Fig. 10).

A better polishing effect after the scaling can be obtained with a dull curette (Fig. 9 and B).







FINISHING OR POLISHING PROCEDURE -

THE FILE -

The using of a cemental file to polish the root surface after a flap operation or a sub-gingival curettage is a less accurate way of polishing the cemental surface and certainly the quickest method to deteriorate that dental tissue by creating numerous excavations and an irregular surface which are not quite suitable for tissue repair or readaptation (Fig. 13 and 14). Polishing, as mentioned above, is most readily obtained in using a slightly dull curette (Fig. 9 and B).

THE CEMENTAL FILE -

To find out to what extent the file is liable to produce disintegration of the cement, we have recorded for comparison, the effect on the cement of the carborundum disk which is considered to be the most powerful grinding substance known in dental art. For the same purpose of comparison we have also used, and rubbed over the roots, a metal disk powdered with fine abrasive (Bird Moyer's). Therefore, next to carborundum, the file is the quickest agent in attacking the cement surface (Fig. 15a and 15B). The finely powdered steel abrasive produces even less marked deterioration than is obtained with the cemental file (Fig. 16a, 16b and 18). But even so, we do not think that this material should be used as a polishing agent.

The hoes first, and secondly, the fine curettes for the scaling, and also some dull curettes for the polishing (with a burnishing effect) seem to be the ideal instruments for the treatment of the cemental wall of the pocket (Fig. B).

C3. CURETTEMENT OF THE BASE OF THE POCKETS -

As was shown in our report no. 1, February 1948, the size of the periodontal curette is too large, in most cases, to make a complete and accurate curettage at the base of the pockets where the pericemental space is so small. In the pathological process, as soon as the gingival crevice begins to be invaded by a downgrowth of epithelium and the products of infection, the volume of the tissue to be curetted is so small at the bottom (fondus) of the pocket that the ordinary curette does not easily reach that tissue except when strong pressure is applied. This gives rise to the danger of proceeding beyond the ulcerated base of the pocket, thus crushing, or damaging important fibres and other vital elements of the periodontal membrane (Fig. 20 and 21).







"L-7"

On account of the disproportion between the size of the curette and the periodontal membrane, we have used clinically for the curetting of the base (fondus) of the pockets, a coarse nerve broach as described in our first report, in order to curette and eliminate the disorganized elements without breaking or damaging the fibres of the membrane.

Biopsy materials obtained from three cases could not be successfully prepared by our technician, as the point of attachment of the periodontal membrane, at the base of the pockets, was too much separated from the cement to record any detail of value.

C4. In planning to carry out our investigations on biopsy materials from autopsy, we intend later on to use, if possible, larger broaches in accordance with the comparison made by means of the following micrographs (Figs. 22, 23).

Signed: "Jules Thébaud"







SOME MICROSCOPIC FEATURES WERE PHOTOGRAPHED  
AS FOLLOWS:

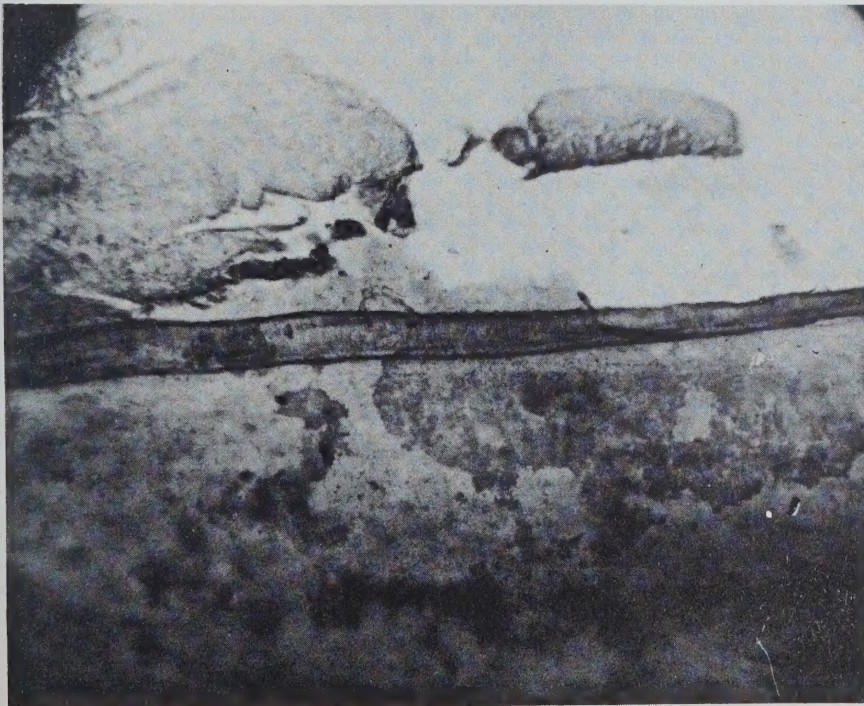


Fig.1 Microphotograph showing normal thickness of untreated (virgin) cemental surface with peridental adhesions and debris.

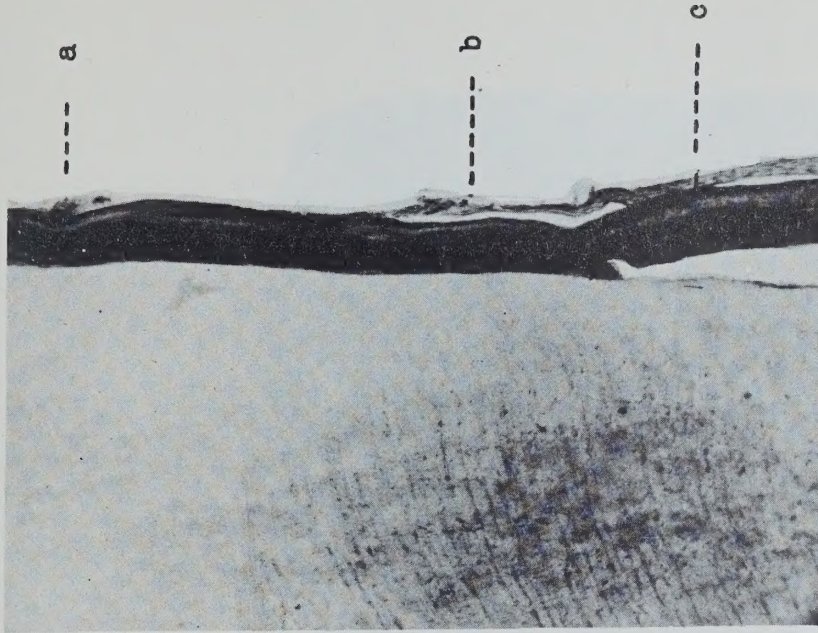
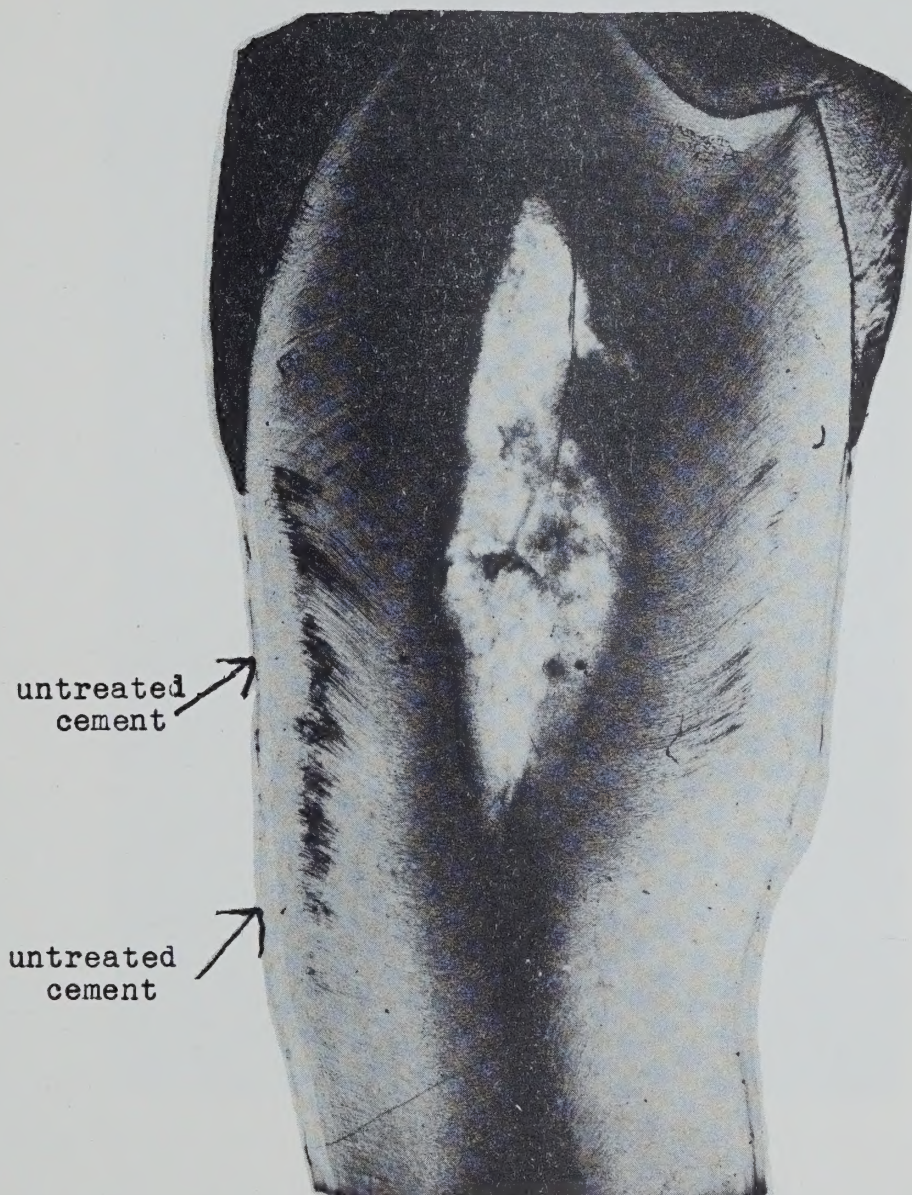


Fig.2 Untreated distal part of a root showing normal thickness of the cement  
(a) debris  
(b) cement cuticula  
(c) detached cemental layer at the gingival third.







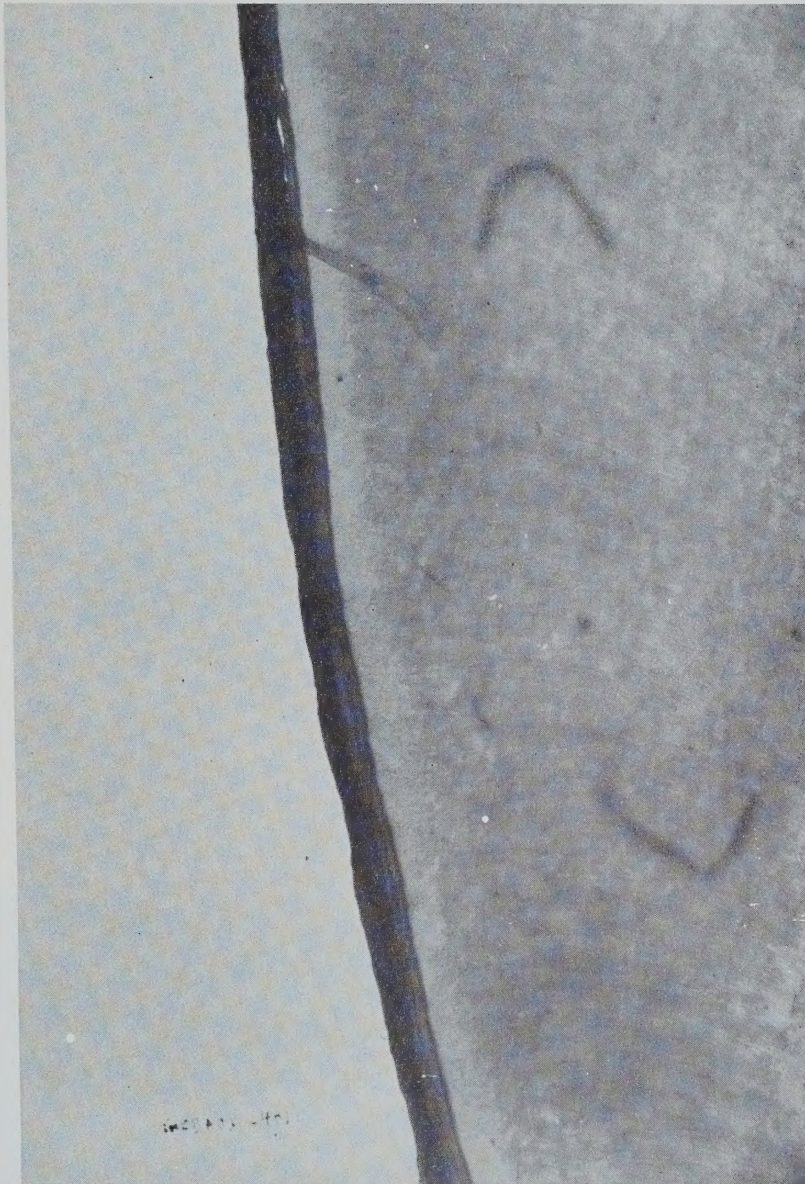


No. 3 Ground sections showing cemental substance slightly thinner after the use of a hoe.









#### ROUTINE PLANING

B Even and clean cut profile of a root curetted with the routine planing technique (hoe, sharp curette used for the scaling and dull curette for the polishing).







untreated  
cement

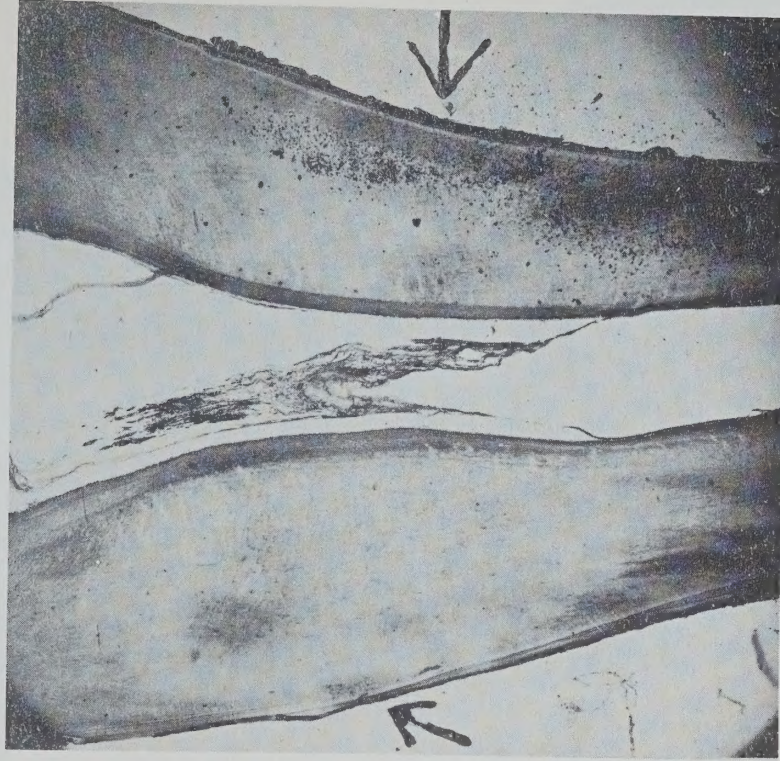
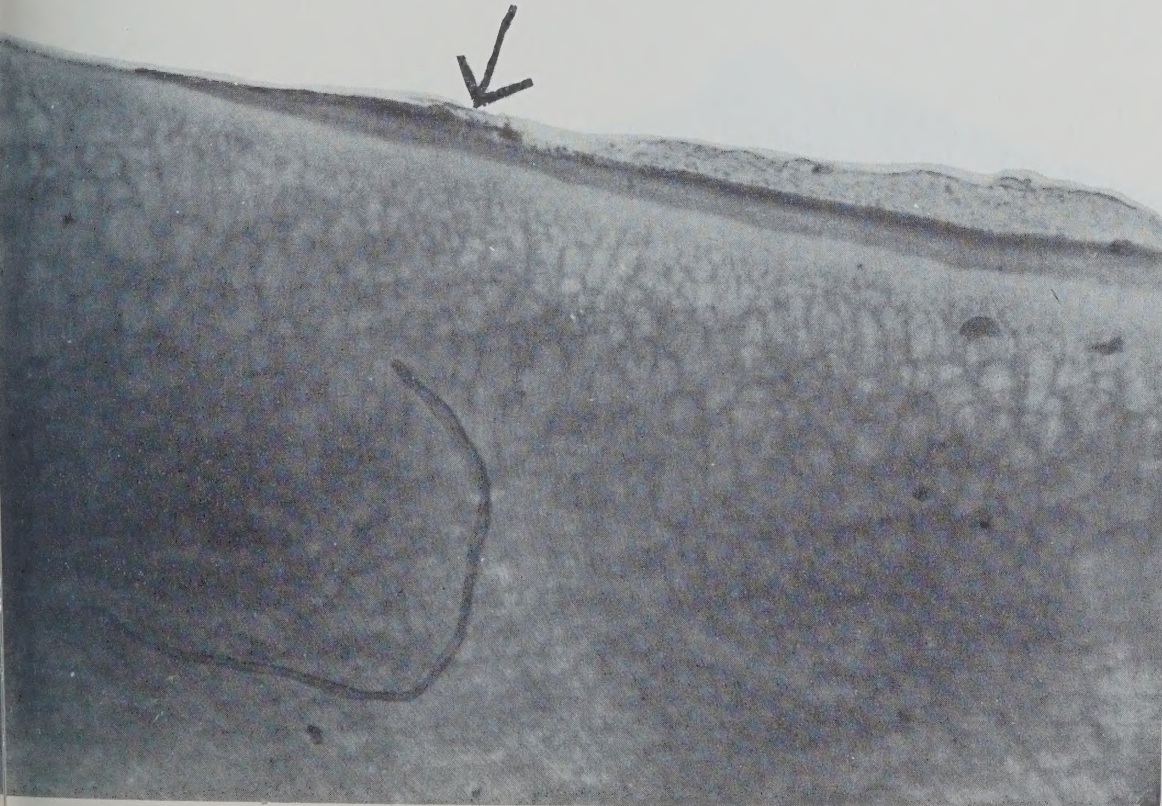


Fig. 4 Cemental surface treated with the hoe only. Note peripheral broken line of cement and small grooved spots.



#### SPOT CURETTAGE

Fig. A Enlarged view of the gingival third of a root where minute particles of concretions were found after a spot curettage.







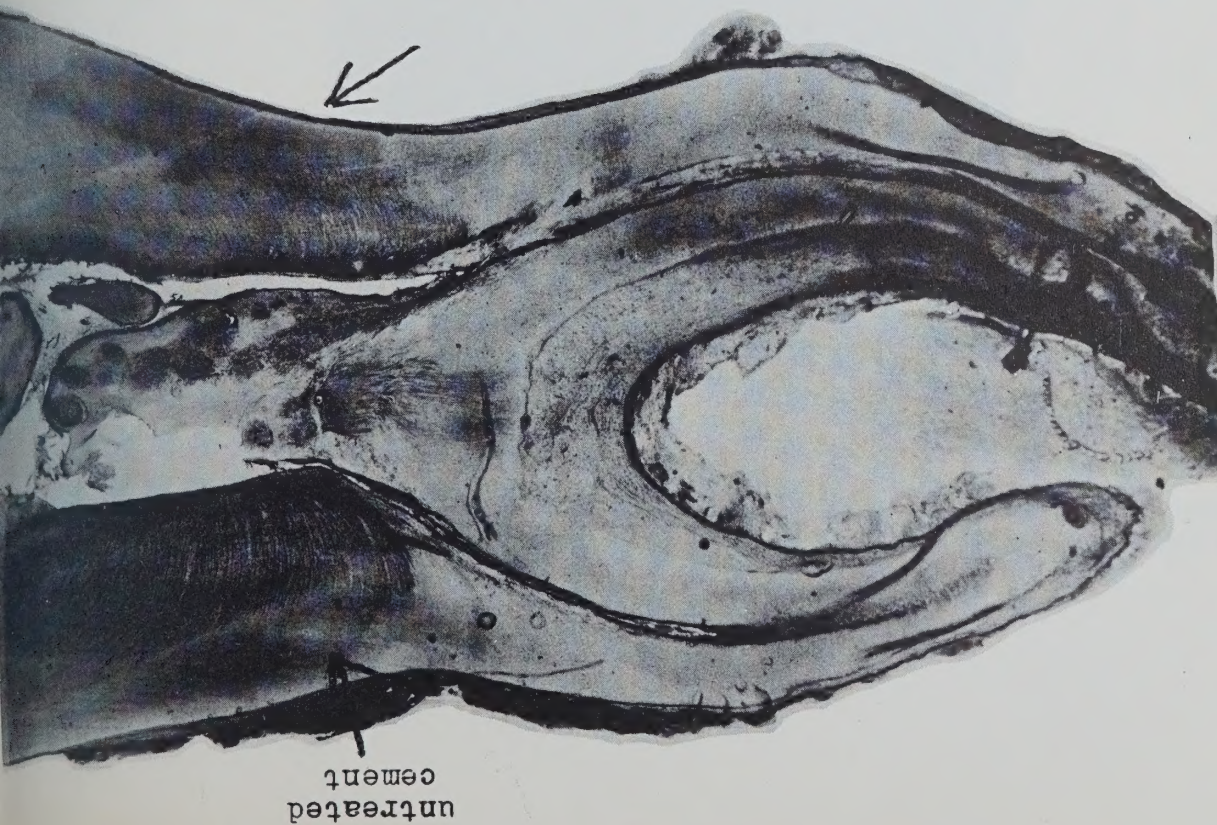


Fig. 5 Grooved gingival cement after the over-use of the hoe.

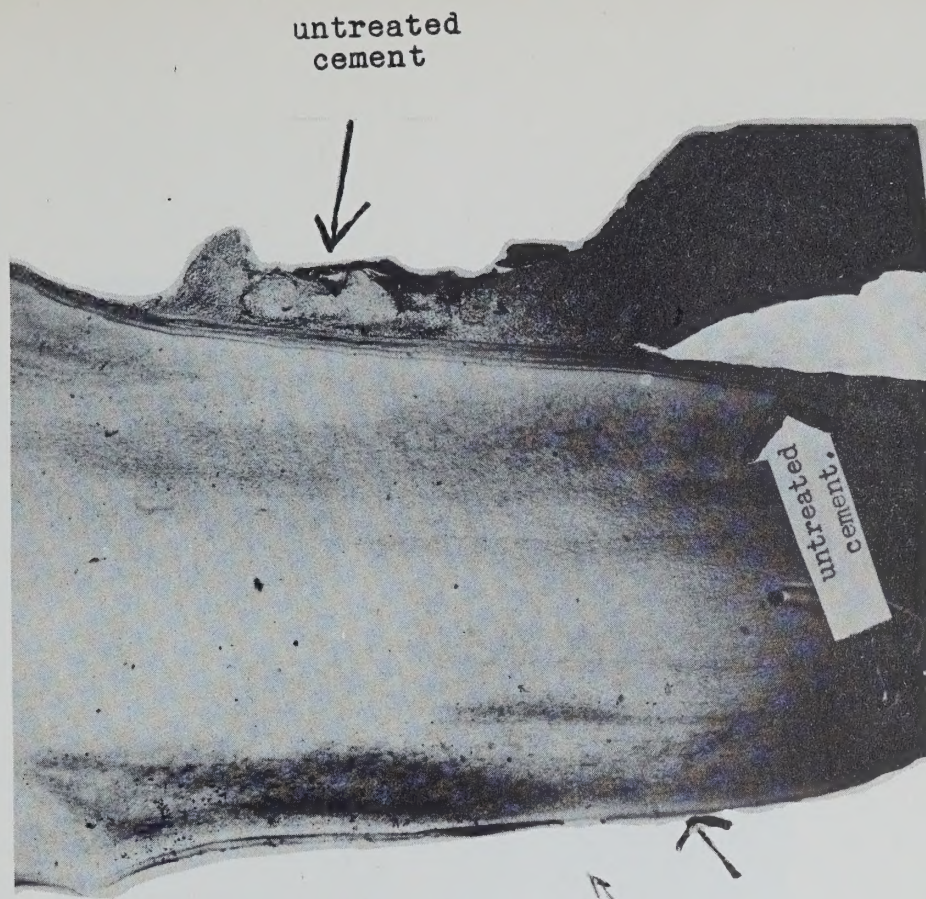


Fig. 6 Irregular line of the cement and exposed dentine following the use of a hoe without proper fulcrum as indicated by Miller (Text Book of Periodontia).







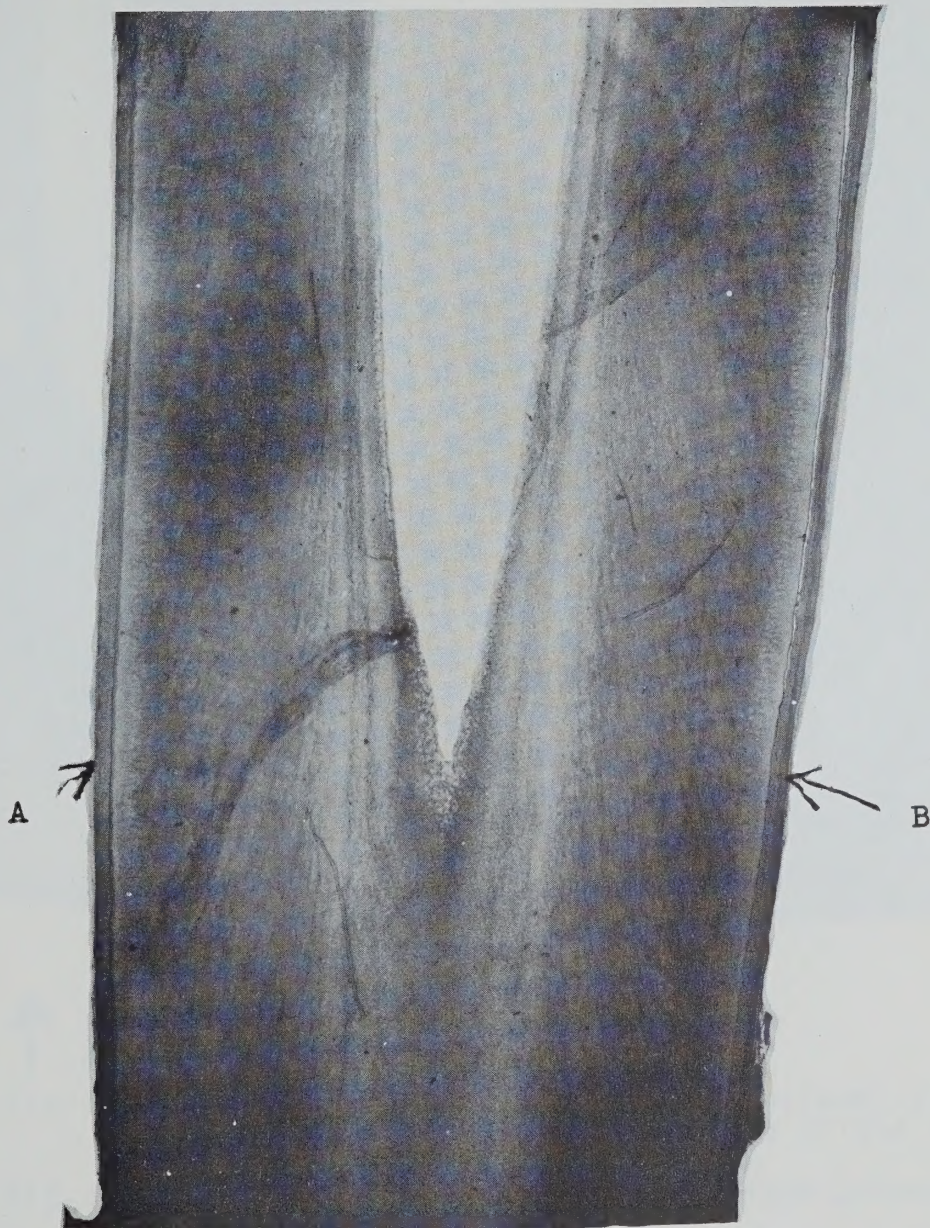


Fig. 7 A - Cement treated with the hoe only.  
B - Polished and slightly thinned cement after  
the combined use of the hoe and the curette.





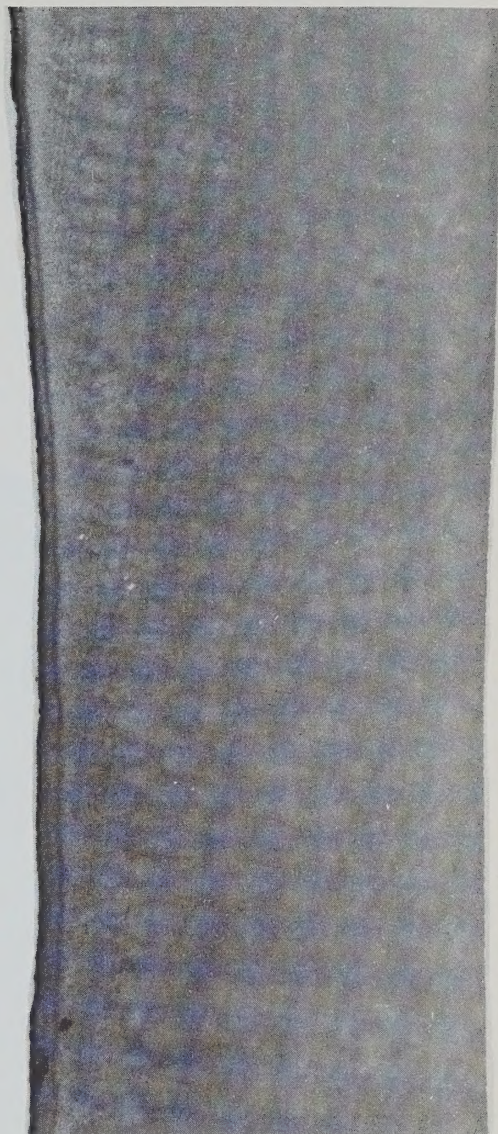
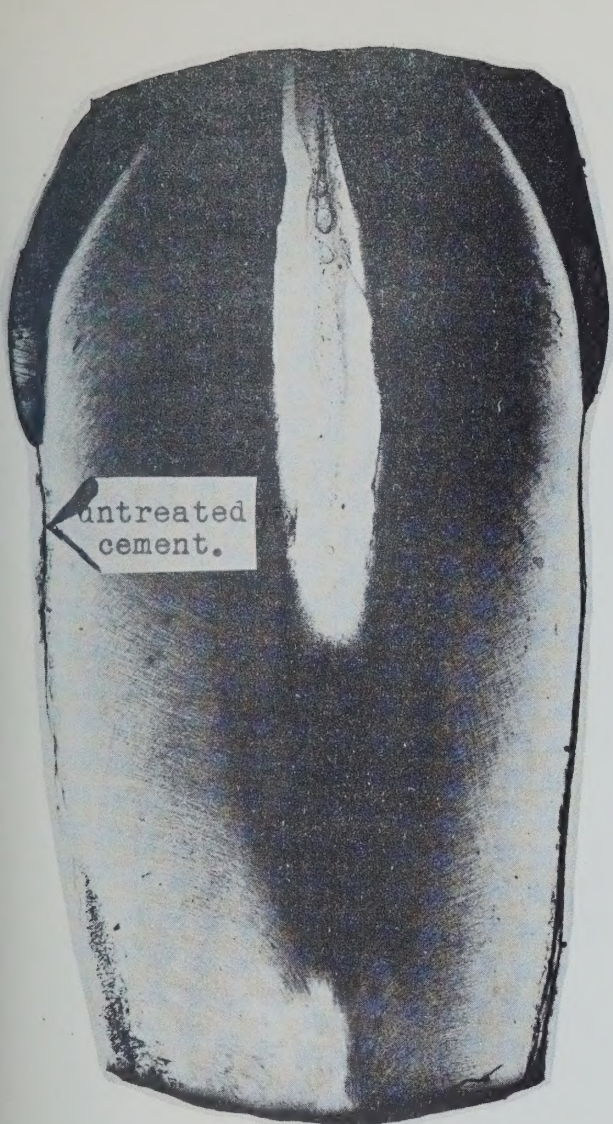


Fig. 8 Ground section showing an even surface of cement following the moderate use of the curette.

Fig. 9 Enlarged view of a cemental surface properly treated with a curette.





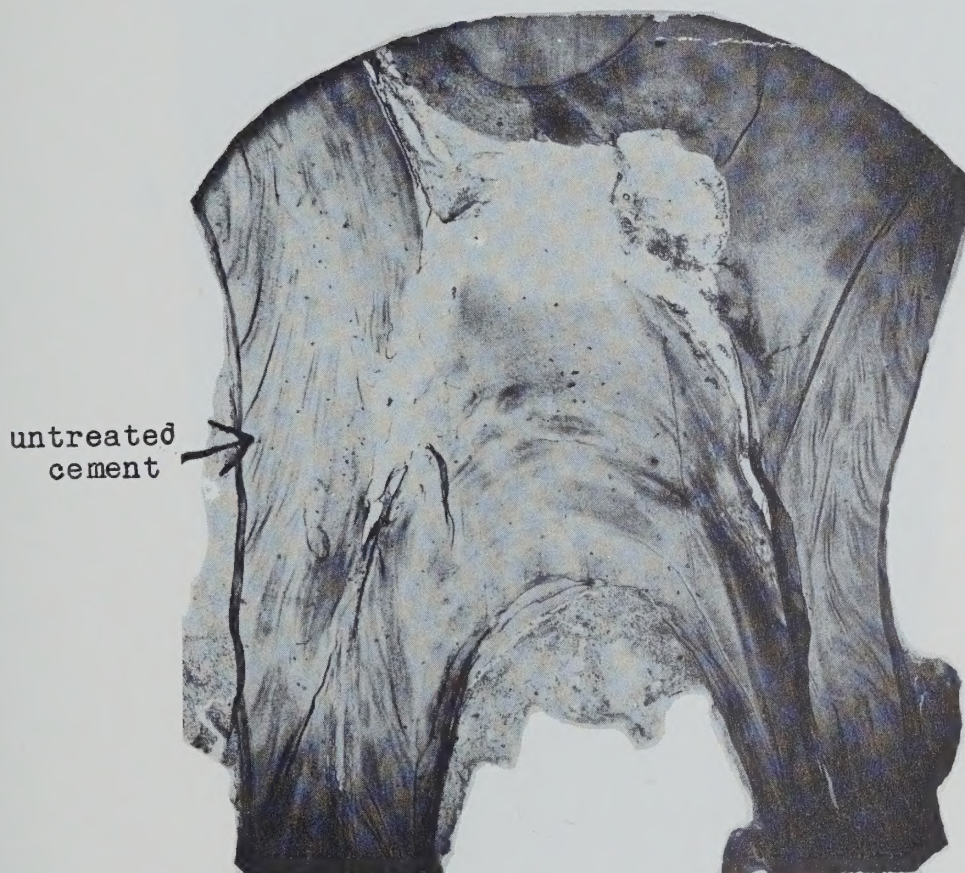


Fig. 10 Microphotograph of a cemental surface treated with a curette over-used (3 curettages performed between intervals of one month).





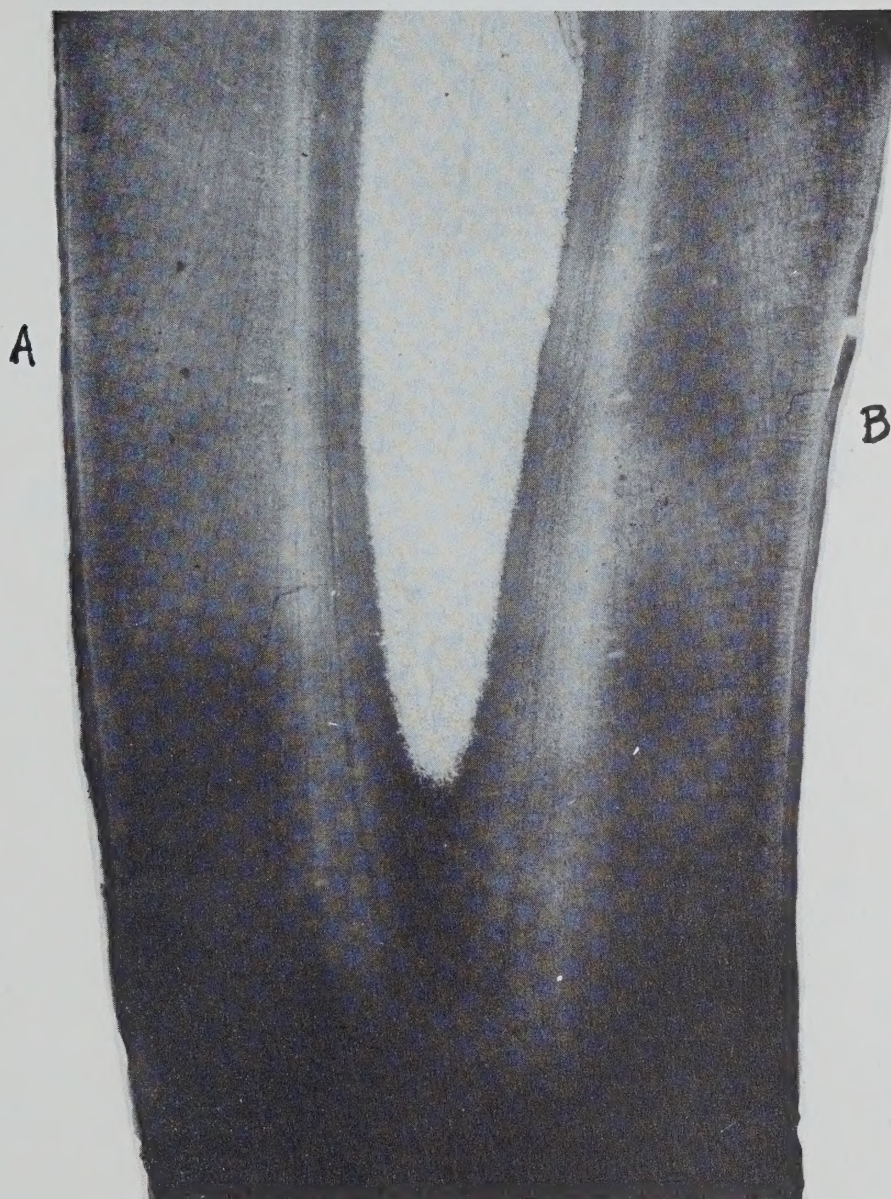


Fig. 11 Difference between: A) Uneven outline of the cement scaled only with the hoe; and B) Regular outline of the cement treated and finished with the curette.





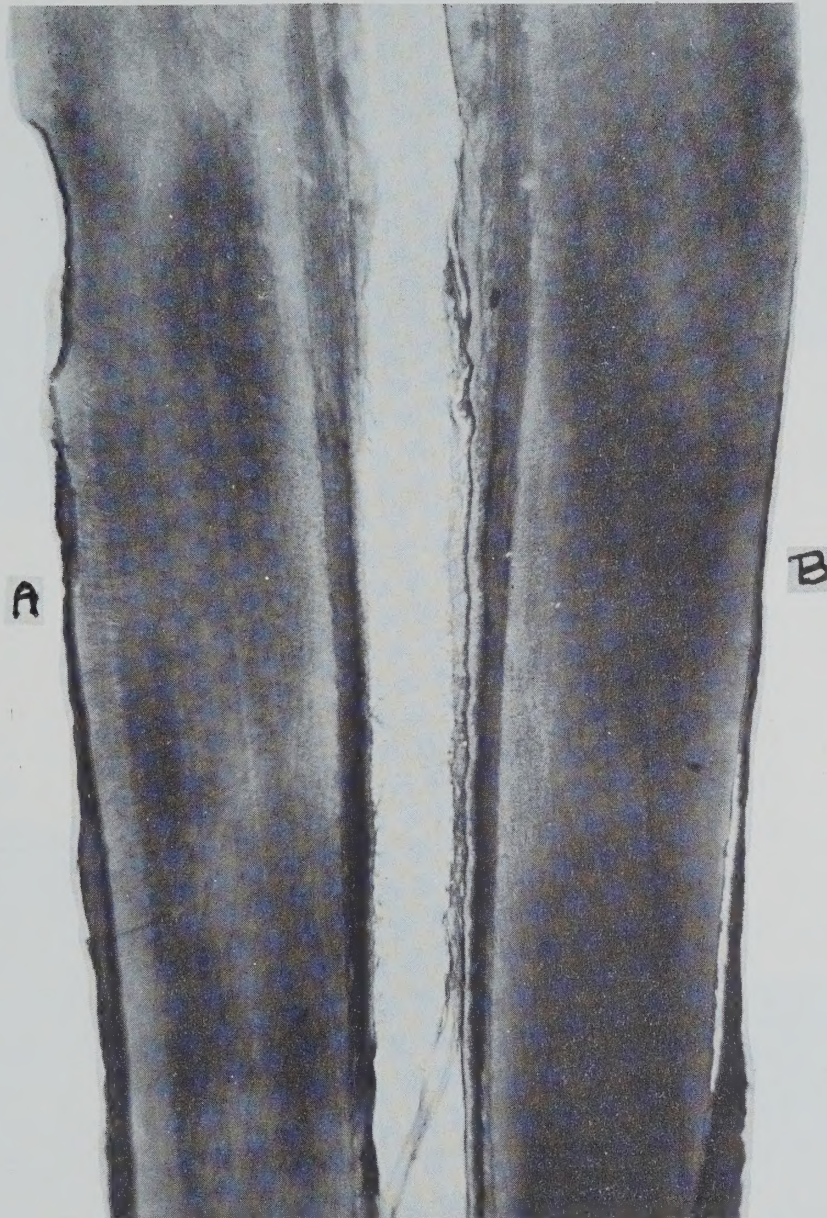


Fig. 12 Difference between: A) Irregular cemental line improperly scaled with the hoe; and B) Cemental surface scaled with the hoe and finished with the curette.





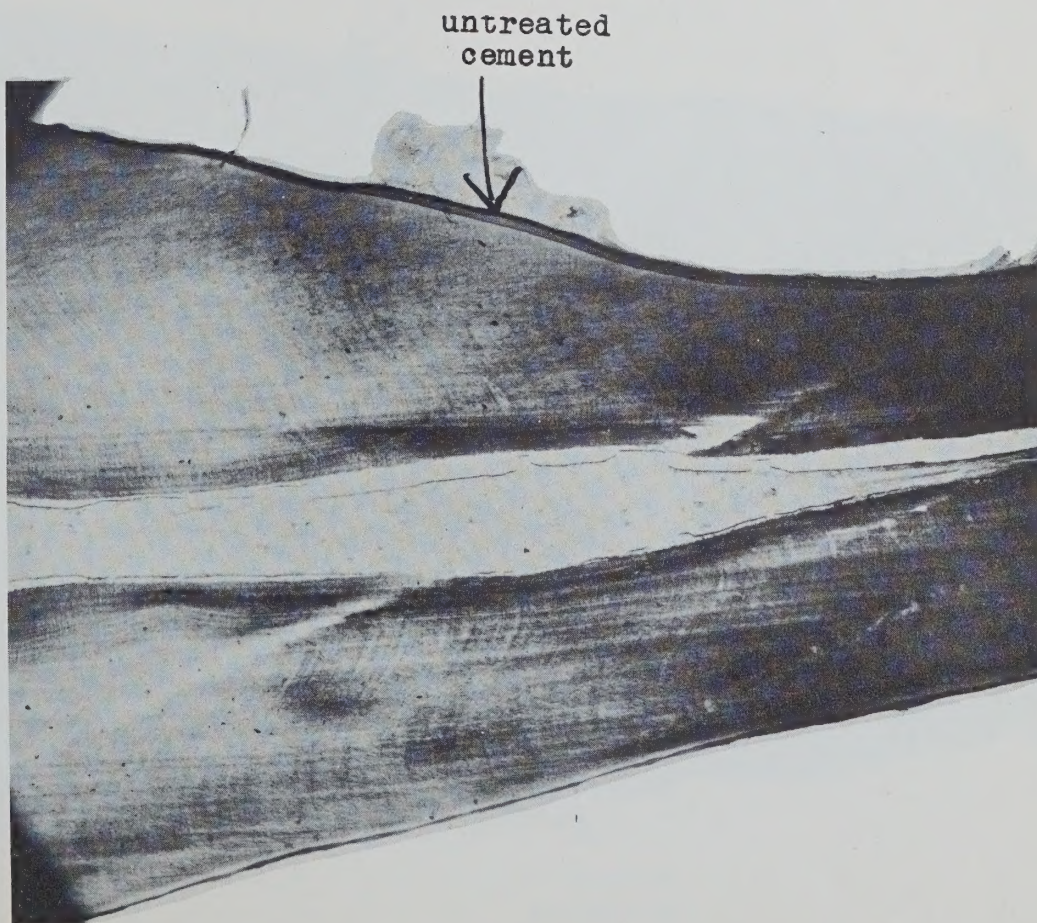


Fig. 13 Outline of a root treated with a file only - Note: irregularity of contour.

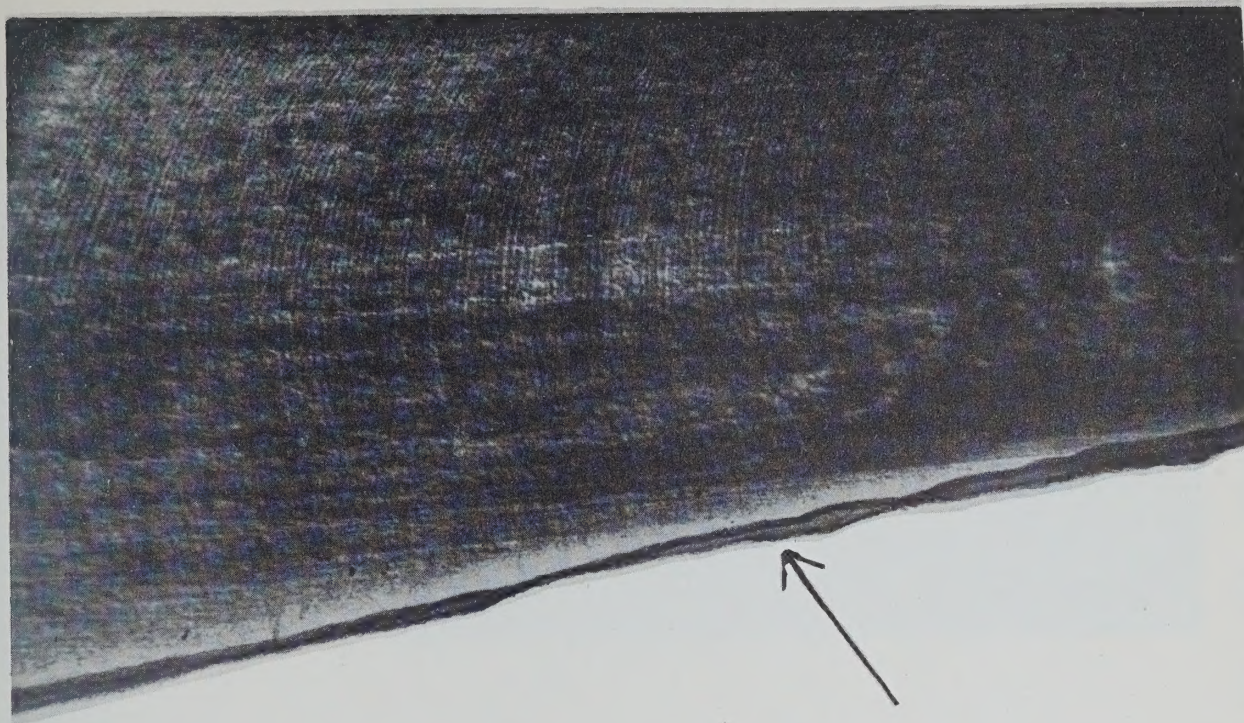


Fig. 14 Enlarged view of an irregular outline caused by the use of the file.







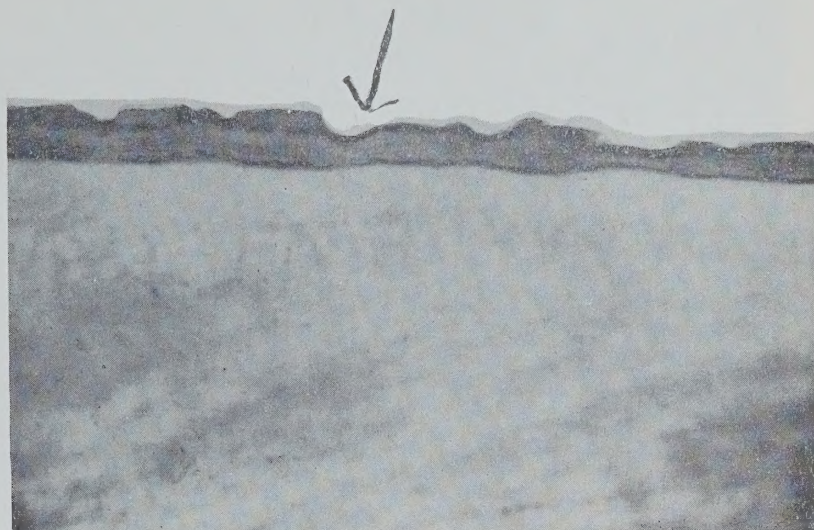


Fig. 15b Enlarged view of a very ragged cemental outline following the rubbing of carborundum disk over the cement.

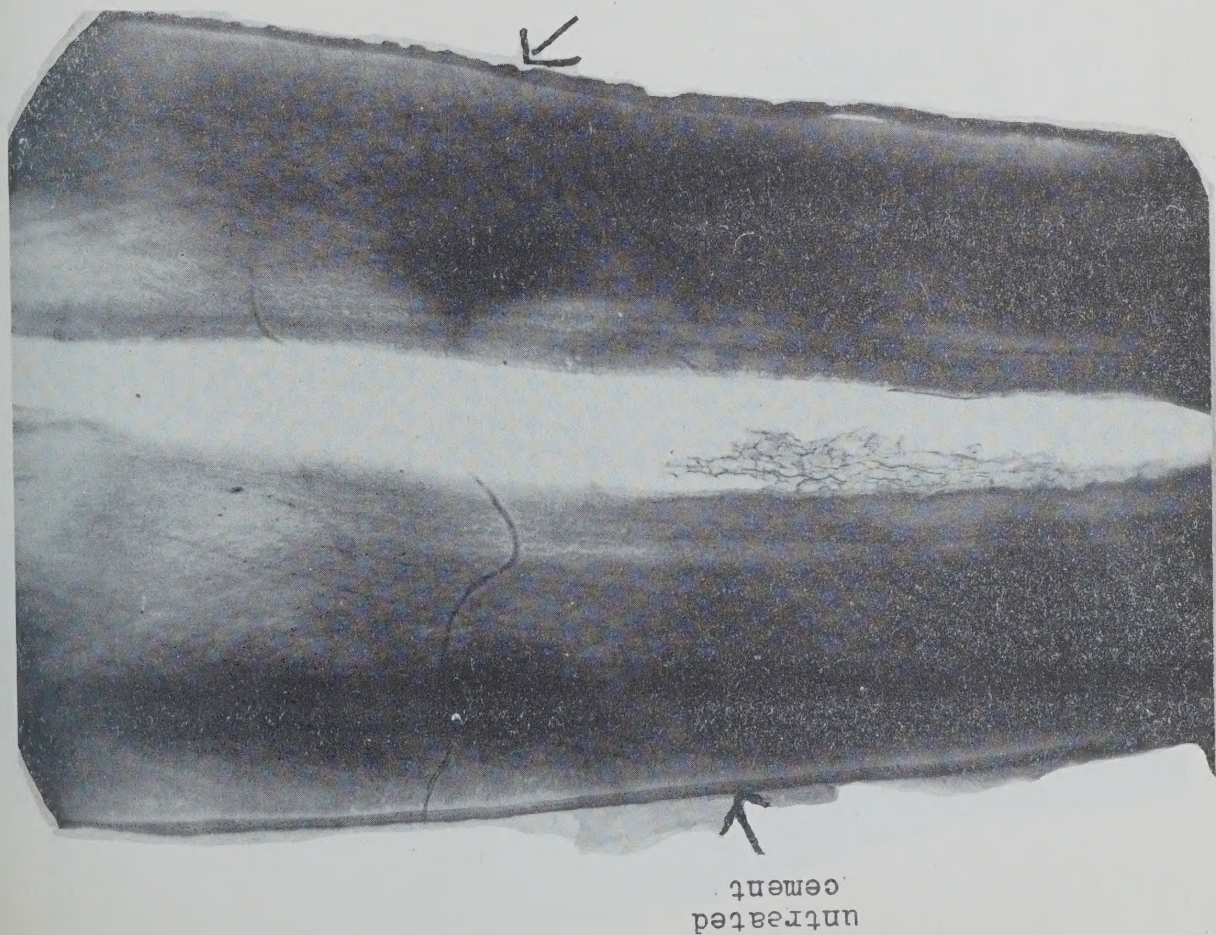


Fig. 15a Very ragged cemental outline following the rubbing of carborundum disk over the cement.

untreated  
cement





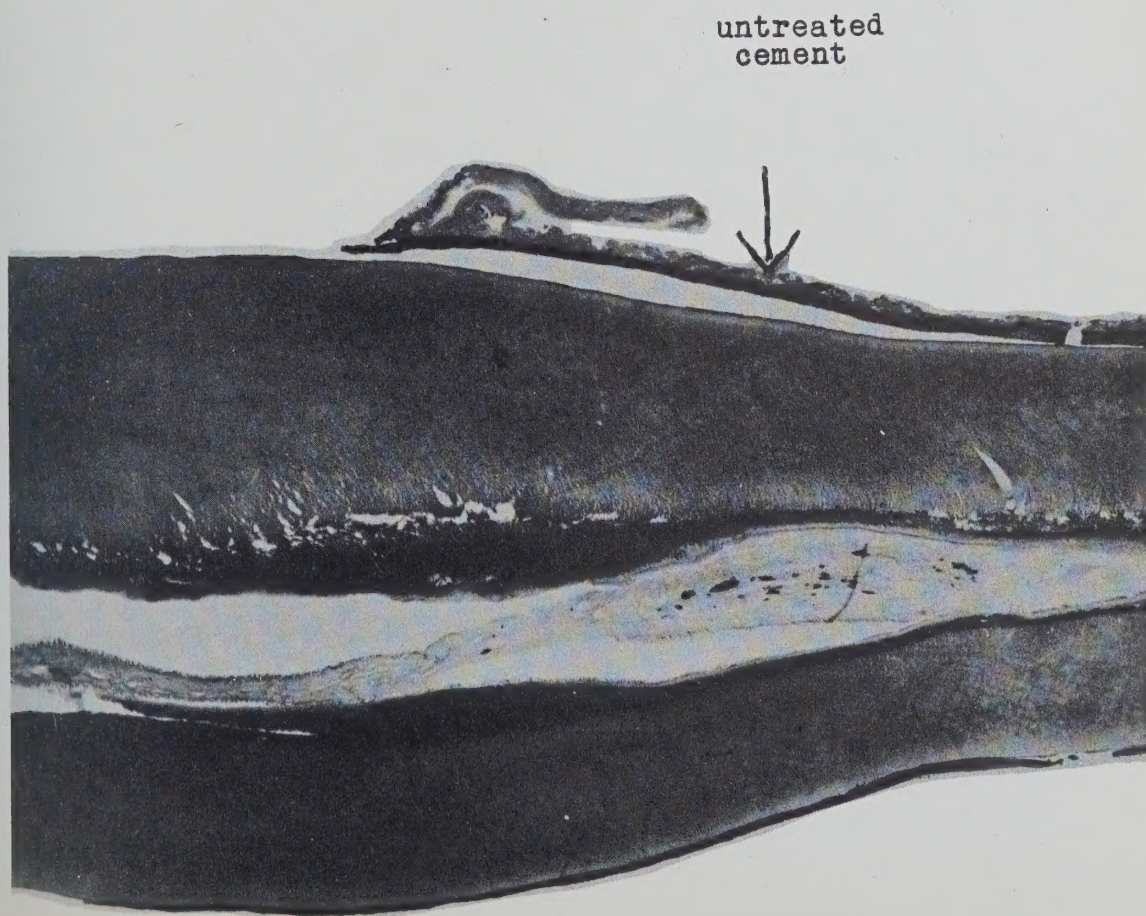


Fig. 16a Uneven and thinned cement as the result of the use of a metal abrasive disk.

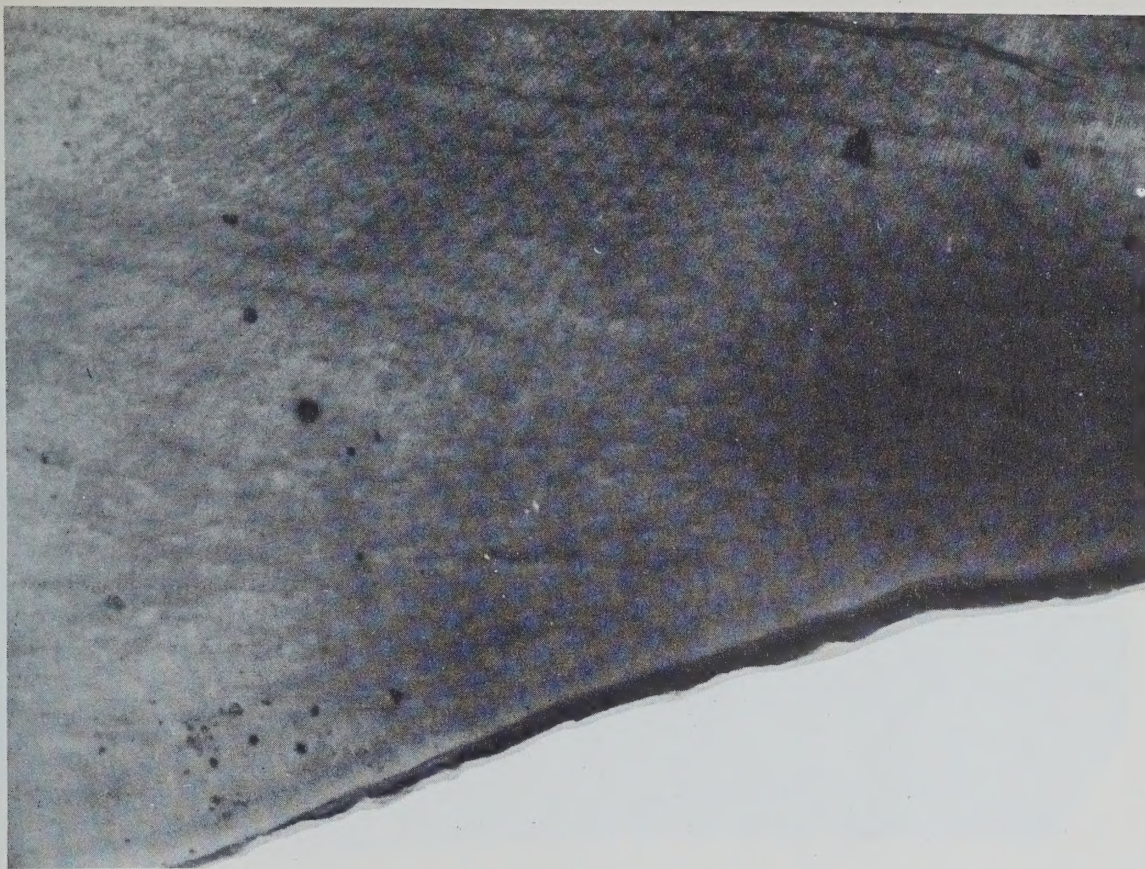


Fig. 16b Enlarged view of uneven and thinned cement as the result of the use of a metal abrasive disk.







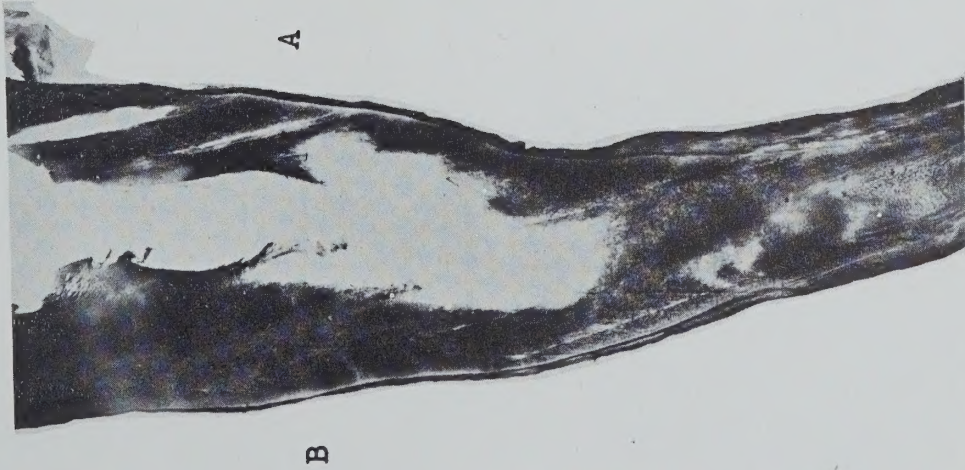


Fig. 17 Comparison between: A) Cement treated with a hoe (no curette); and B) Cement scaled with a hoe and finished with a file. - Note thinness and irregular outline.

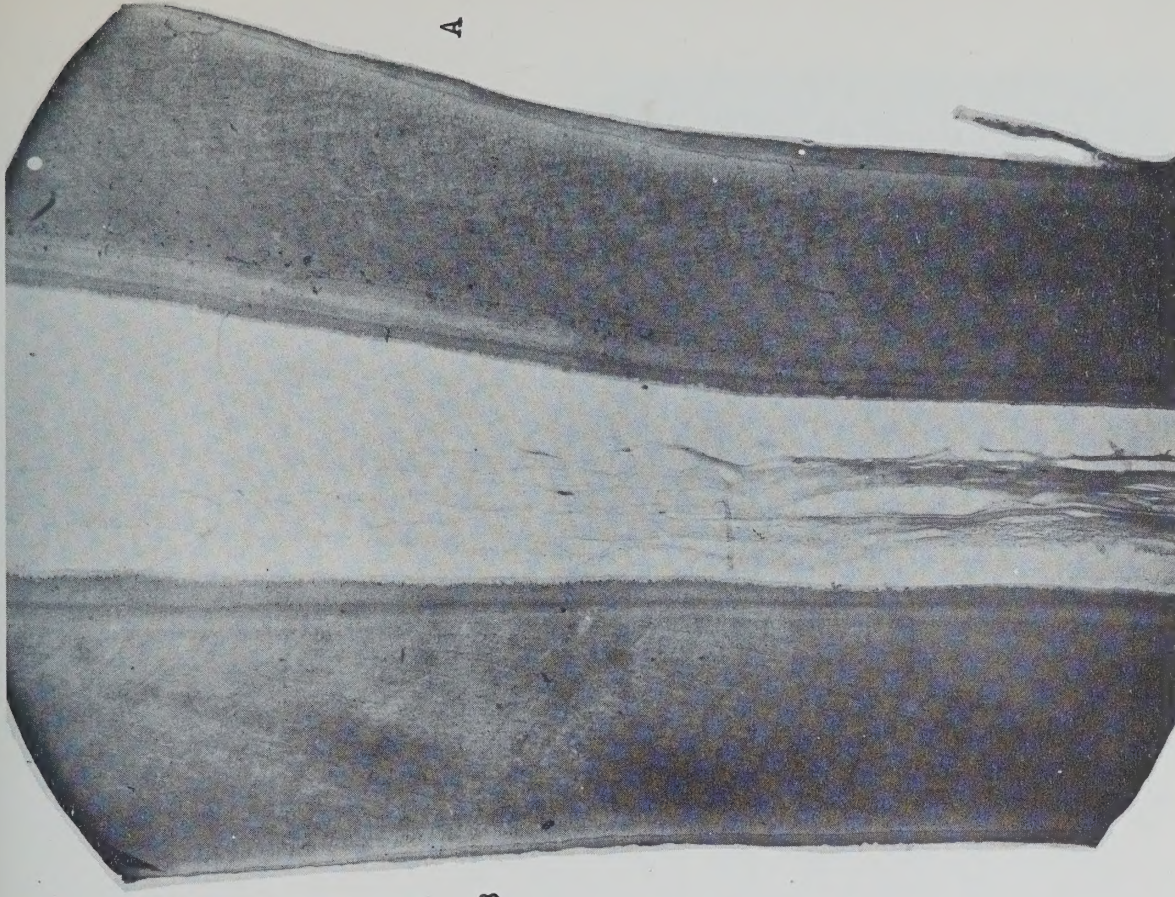
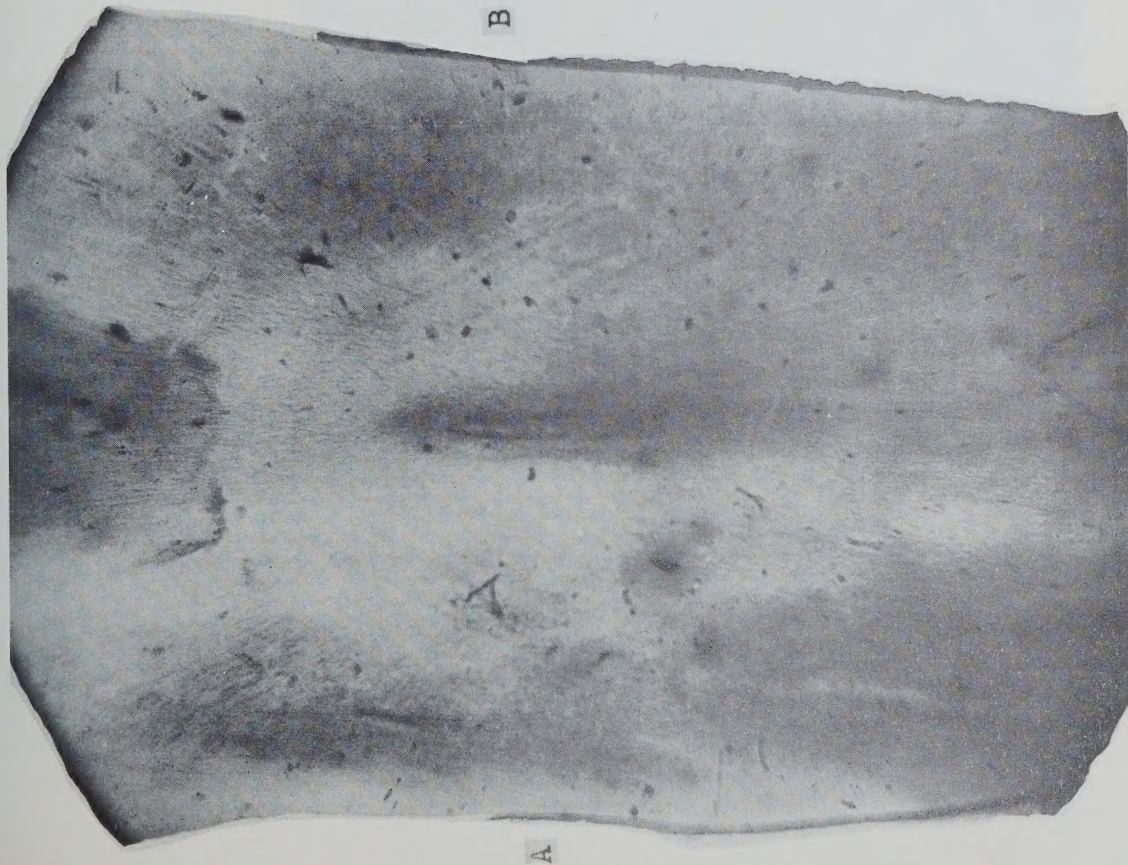


Fig. 18 Comparison between: A) Part of the root treated with a curette; and B) Portion of the root scaled with a curette and polished with abrasive steel.







B



Fig. 19 A) Outline of the cement treated with a hoe and a curette.  
B) Very thinned and uneven cement scaled with a curette, a carborundum disk.

Fig. 20 Position of a periodontal probe reaching the base of a pocket.





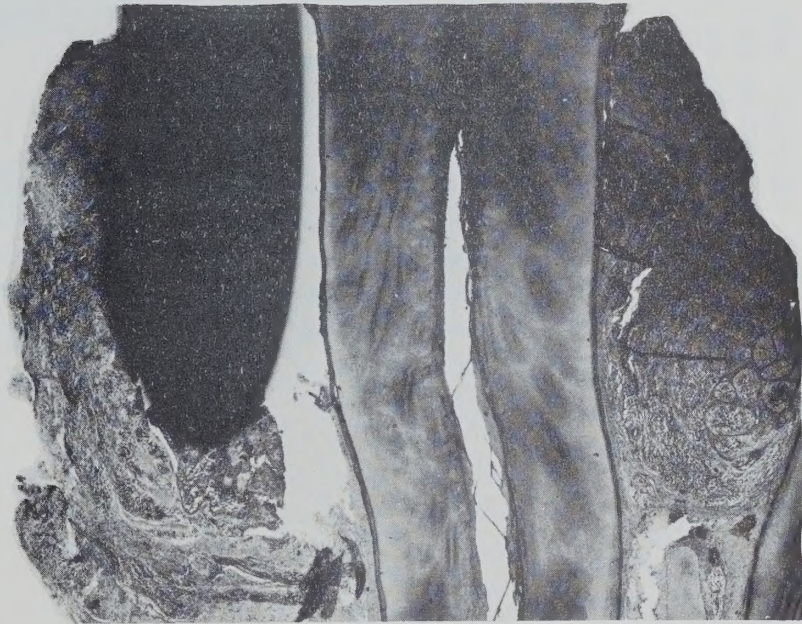
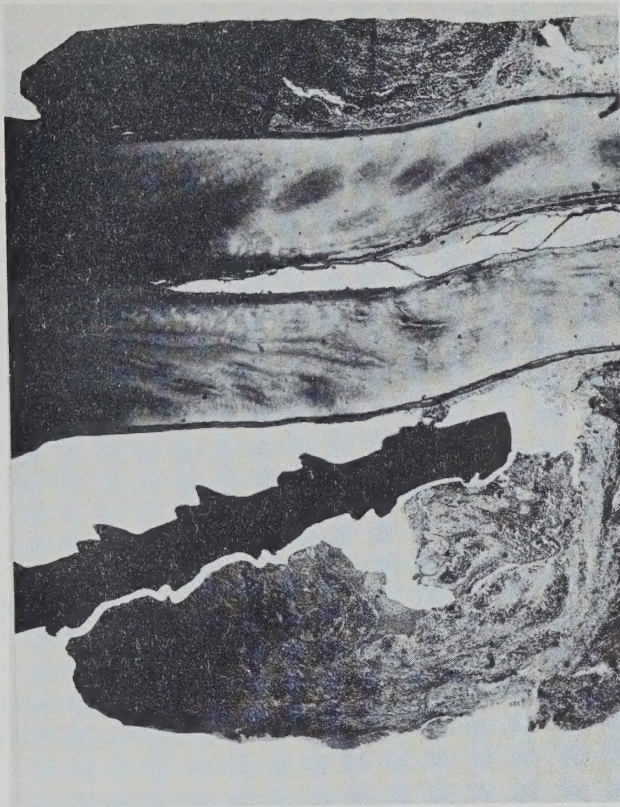
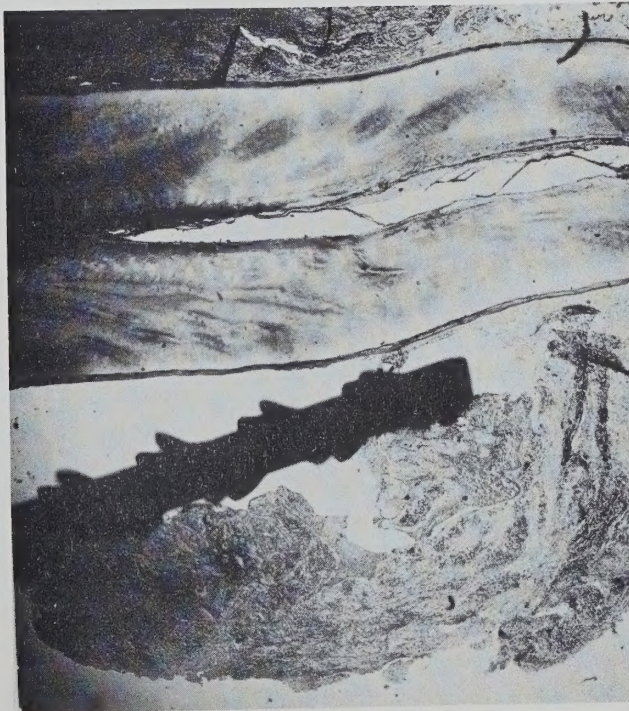


Fig. 21 Position of a McCall's curette toward the base of a pocket. Compare the size of the curette with the size of the tooth.







Figs. 22,23 Position of a nerve broach reaching freely the base of a pocket. Note difference in size between the curette and the broach.





# APPENDIX "M"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: Biological Absorption of Fluorine  
 Grantee: Dr. M.D. Smith  
 Project: DR 29 Amount of Grant: \$1,500

### SUMMARY OF WORK

The completion of studies under DR 14 as well as work on DR 29 are reported.

In a continuation of work begun in the summer of 1948, vegetables were grown where the fluorine content of the atmosphere was believed high (glass factory) and where it was lower.

| <u>Vegetables</u> | <u>Fluoride content(p.p.m. dry wt.)</u> |             |                      |             |
|-------------------|-----------------------------------------|-------------|----------------------|-------------|
|                   | <u>Ave. Rd. &amp; Bloor</u>             |             | <u>Glass Factory</u> |             |
|                   | <u>1948</u>                             | <u>1949</u> | <u>1948</u>          | <u>1949</u> |
| Spinach           | 112, 117                                | 60.2, 56.4  | 154, 169             |             |
| Lettuce           |                                         | 73.7, 52.2  |                      |             |
| Beet greens       |                                         | 34.3, 36.3  |                      | 36.3, 36.2. |
| Beets             |                                         | 0 0         |                      | 0.6, 0.2    |

Poplar leaves were analyzed for fluoride at various periods this season with the following results:

May, 17.7, 18.7 p.p.m.  
 Aug. 19.6, 13.0 p.p.m.  
 Oct. 86.0, 79.2 p.p.m.

A number of foods and beverages were analyzed for their fluoride content. Since baking powders are an important source of fluorides, they were included. Both a single distillation method (Godfrey & Shrewsbury, J.A.O.A.C., 28:335, 1945) and a double distillation method (Methods of Analysis of A.O.A.C., 1945) were attempted with phosphate baking powder with rather inconclusive results. A single and a double distillation procedure were also carried out with the combination baking powder and the results seemed to justify the use of a double distillation with this type.



(SUMMARY)

| <u>Substance</u>                               | <u>Fluorine content</u>           |
|------------------------------------------------|-----------------------------------|
| Coffee infusion                                | 0.88 micrograms fluorine /100 ml. |
| Toronto water                                  | 0.12, 0.11 p.p.m.                 |
| Coca-cola                                      | 0.06, 0.07 p.p.m.                 |
| Breakfast (juice, cream of wheat, toast, mar.) | 1.04, 0.77 p.p.m (dry weight)     |
| Dinner - salad meal                            |                                   |
| - beef, macedoine, etc.                        | 0.11, 0.26 p.p.m.                 |
| - halibut, spinach, "                          | 1.04, 0.92 p.p.m.                 |
| Pabena (baby food)                             | 11.3, 12.1 p.p.m.                 |
| Bonemeal (capsules)                            | 246, 285, p.p.m.                  |
| Soluble fluorine in Pabulum                    | app. 10% at 40°C. for 15 min.     |
| Royal B.P. - (tartrate)                        | 1.36, 1.34 p.p.m.                 |
| Magic B.P. - (phosphate)                       |                                   |
| single distillation                            | 6.0, 6.1 p.p.m.                   |
| Double distillation                            | 5.9, 14.0 4.7, 0.84 p.p.m.        |
| Calumet B.P. - (combination)                   |                                   |
| single distillation                            | 19.1, 12.4, 15.3, 16.4 p.p.m.     |
| double distillation                            | 25.0, 32.0 p.p.m.                 |

The method of determining fluorides has been adapted to urine and is being investigated still further. Preparations have been made for fluoride balance studies on infants, but the first attempt was unsuccessful owing to difficulty with the urine adapter. Suitable subjects have now been obtained and balance studies with and without Pabulum in the diet are under way.

Experiments to measure the amount of fluorine in the atmosphere by bubbling the air through dilute sodium hydroxide, over solid sodium hydroxide and granular magnesium oxide were unsuccessful, even when sodium fluoride or potassium fluo-silicate in acid solution was heated in an oven to 140°C. and the resultant atmosphere passed through the absorbing system.

(SIGNED:) M. Doreen Smith



# APPENDIX "M"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: Biological Absorption of Fluorine  
 Grantee: Dr. M.D. Smith  
 Project: DR 29 Amount of Grant: \$1,500.

This report includes results obtained in completion of a study of the fluoride content of various foods begun under Project DR 14 as well as work on Project DR 29.

In continuation of work begun in the summer of 1948, vegetables were grown in the same soil in an area where atmospheric fluoride was believed high (glass factory) and one where it was lower. Chiefly because of an unfavourable growing season, samples were not obtained in all cases as desired.

| <u>Vegetables</u> | <u>Fluoride content(p.p.m. dry wt.)</u> |             |                      |             |
|-------------------|-----------------------------------------|-------------|----------------------|-------------|
|                   | <u>Ave. Rd. &amp; Bloor</u>             |             | <u>Glass Factory</u> |             |
|                   | <u>1948</u>                             | <u>1949</u> | <u>1948</u>          | <u>1949</u> |
| Spinach           | 112, 117                                | 60.2, 56.4  | 154, 169             |             |
| Lettuce           |                                         | 73.7, 52.2  |                      |             |
| Beet greens       |                                         | 34.3, 36.3  |                      | 36.3, 36.2  |
| Beets             |                                         | 0 0         |                      | 0.6, 0.2    |

Although seed and soil were the same in both districts, the spinach and lettuce did not grow at the glass factory. No conclusions are drawn as to the cause of this failure. The beet greens in both districts had a similar fluoride content and there was negligible fluoride in the roots.

Poplar leaves were also analyzed at different periods last season to see if the fluoride content was increasing as suggested by Churchill et al, (J. Ind. Eng. Chem., anal. 20:69, 1949).



|               | Fluoride content (p.p.m. dry wt.) |            |            |
|---------------|-----------------------------------|------------|------------|
|               | May, 1949                         | Aug. 1949  | Oct. 1949  |
| Poplar leaves | 17.7, 18.7                        | 19.6, 13.0 | 86.0, 79.2 |

There appears to be an increase in the fluoride content of the leaves chiefly in the latter part of the growing season.

Baking powders are an important source of fluoride and in Canada a maximum of 1.4 p.p.m. fluoride in a cooked food where baking powder is used, has been considered. In Britain, baking powders may not contain more than 100-130 p.p.m. fluoride. The determination of fluorides in phosphate baking powders involves a double distillation with perchloric acid, (Methods of Analysis of the Association of Official Agricultural Chemists, 1945), since the phosphates may interfere with the titration to give high results. However, Godfrey and Shrewsbury (J.A.O.A.C., 28:335, 1945) recommend a single distillation for the determination of fluoride in minerals and this was also attempted. The determination of fluorides in combination baking powders containing aluminum, which represses the evolution of fluorine, necessitates a preliminary distillation with sulphuric acid followed by a distillation with perchloric acid. Single distillations were also attempted on this type.

| <u>Baking powder</u>  | <u>Fluoride content, p.p.m.</u> |
|-----------------------|---------------------------------|
| Royal - tartrate      | 1.4, 1.3                        |
| Magic - phosphate     |                                 |
| single distillation   | 6.0, 6.1                        |
| double distillation   | 5.9, 14.0, 4.7, 0.8             |
| Calumet - combination |                                 |
| single distillation   | 19.1, 12.4, 15.3, 16.4          |
| double distillation   | 25.0, 32.0                      |

The results with the phosphate baking powder are inconclusive and seem to indicate an unreliability of the method with this substance. A double distillation method with the combination baking powders seems advisable.

A number of foods and beverages were analyzed. A coffee infusion was quite low in fluoride - 0.88 microgm. fluoride per 100 ml. - as compared with tea infusions which were quite high in fluoride - 91.6-127.0 microgm. per 100 ml. Toronto water had a fluoride content of 0.12, 0.11 p.p.m. and coca-cola 0.06, 0.07 p.p.m.



A breakfast including orange juice, cream of wheat, toast and marmalade, a lunch including fruit salad and dessert and a hot dinner with beef, potatoes, macedoine vegetables and dessert were analyzed and compared with a fish dinner, including potatoes, spinach and dessert already reported.

| <u>Meal</u>          | <u>Fluoride content</u> |                   |
|----------------------|-------------------------|-------------------|
|                      | <u>p.p.m.</u>           | <u>Total mgm.</u> |
| Breakfast            | 1.04, 0.77              | app. 0.10         |
| Dinner - salad, etc. | - -                     | -                 |
| beef, etc.           | 0.11 0.26               | app. 0.03         |
| halibut, etc.        | 1.04, 0.92              | app. 0.15         |

Pabena, a baby food similar to Pablum was also found to have a high fluoride content - 11.3, 12.1 p.p.m. Bonemeal capsules, "Bone-aid", obtained from the research department of Wayne University were found to contain 246, 285 p.p.m. fluoride.

It had been suggested by Dr. Rae at the ninth meeting of the Associate Committee on Dental Research that the determination of water-soluble fluorides as distinct from calcium fluoride should be made. It was found that the fluoride in Pablum - (app. 12 p.p.m.) is app. 10% water soluble at 40°C. for 15 min. It seems that at high levels of intake, calcium fluoride is far less toxic to the rat than sodium fluoride (Smith and Leverton, J. Ind. Eng. Chem., 26:791, 1934) but at low levels of intake such as are usual in foods, the form in which fluoride exists in the food probably makes little difference to its absorption and toxicity (Smith and Leverton, J. Ind. Eng. Chem. 26:791, 1934, Lawrenz et al., Journal of Nutrition, 18:115, 127, 1939). However, Mackle and Largent (J. Ind. Hyg. and Toxicol., 25:112, 1943) and McClure et al., (J. Ind. Hyg. and Toxicol., 27:159, 1945) have found that at low levels of intake app. 60% of the fluoride in calcium fluoride in food and 40% of that in bonemeal is absorbed from food as compared to approximately 90% from sodium fluoride in food.

In preparation for the work on the absorption of fluoride by humans, especially children, a number of fluoride analyses of urine were completed. Experiments resulted in high acid distillates which had to be discarded, but on the addition of approximately 10 gm. of silver sulphate to precipitate the chlorides, the acidity of the distillate was lowered to an acceptable level. However, the amount of precipitate in the distilling flask rather complicates the distillation and techniques for removing this excess precipitate are being investigated.



Preparations have been made for fluoride balance studies on infants. In the first experiment, collection and analyses of 24 hr. samples of excreta from the mother before the birth of the child were made. However, the attempt at collection was unsuccessful with the infant due to difficulty with the urine adapter. Through contact with the Infants' Home in Toronto, subjects have now been obtained which should be more suitable. Balance studies with and without Pabulum in the diet are under way.

Since it was felt that the method of measuring atmospheric fluorine was not satisfactory (see previous report) further work was done on this aspect of the problem. The absorption agent used was 0.5N NaOH (Enamelist, p.5, Jan. 1949) and a method of bubbling a known amount of air through this agent to give an accurate measurement of the fluorine content of the atmosphere was devised with the assistance of Dr. Katz, Defence Research Board, Ottawa. In numerous experiments, there was, however, no appreciable absorption of fluorine from the atmosphere by this agent. Solid magnesium oxide was also used as an absorption agent in the assembly, but again there was no indication that fluorine was taken up. In a number of experiments, fluorine as sodium fluoride or potassium fluosilicate solution was added to 1:1 sulphuric acid, placed in a hot air oven and heated to 140° C. or above (the temperature of distillation of fluorine). The air was continuously removed from the oven by suction and passed through dilute sodium hydroxide solution, solid sodium hydroxide and granular magnesium oxide. In no case was the fluoride content of these agents increased, yet the fluorine was no longer present in the acid solution. To obtain an agent capable of removing atmospheric fluorine poses a problem, especially since the form or forms in which it occurs in the atmosphere are largely unknown.

(SIGNED:) M. Doreen Smith



APPENDIX "N"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario.

Grantee: M. Doreen Smith

Project: DR 13 Amount of Grant: \$1,475

SUMMARY OF WORK

The fluorine content of teeth extracted from individuals living in Kitchener, a low fluoride area and Stratford, a comparatively high fluoride area has been studied. Fluorine determinations were made on whole teeth using the modified Willard and Winter technique. In general it would appear that Stratford teeth have a higher fluorine content than Kitchener teeth. The trend for the fluorine content to increase with age was also noted. Differences between results for sound and carious teeth could not be correlated.

Analyses were also carried out on teeth from young dogs who had been treated with topical applications of 2% sodium fluoride. An average increase in the fluorine content of the teeth from .032 to .099 per cent was noted after 8 treatments at weekly intervals.

The Manly and Hodge technique for separating teeth into enamel and dentin fractions was applied to a number of teeth extracted from Brantford children. The separated fractions were analyzed for fluorine content by the chemical method. The ratio of fluorine in dentin to fluorine in enamel was estimated to be 2.7 for permanent teeth and lower for deciduous.

Work on the microbiological technique of fluorine analysis has also been carried on, an attempt having been made to apply the method to samples of urine. The results are more promising for urine than were previously obtained with teeth. Further work is required to increase the accuracy of the method.

(SUMMARY)

Also a method of fluorine analysis using an enzyme (lipase) rather than the microorganism was examined. Only preliminary experiments were made but a comparison of the two methods indicated that the enzyme method would be no improvement on the microbiological.

(SIGNED:) M. Doreen Smith



## APPENDIX "N"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: A study of methods of fluorine analysis to be applied to extracted teeth from people living in different parts of Ontario

Grantee: M. Doreen Smith

Project: DR 13 Amount of Grant: \$1,475

Fluorine Content of Teeth from High and Low Fluoride Areas

Because of the possible significance of the fluorine content of teeth to their resistance to dental caries, a study was made of teeth extracted from individuals living in relatively high and low fluoride areas. The cities of Stratford (1.25 to 1.33 p.p.m. fluorine in municipal water supply) and Kitchener (0.12 to 0.19 p.p.m. fluorine in water supply) were chosen as representative areas so that the work might be correlated with a study previously made of the fluorine content of water and milk from these two communities. Teeth from two other areas have also been analyzed and the results included for purposes of comparison. These are Ripley, Ont., reported by Box and Hodgins (1) as a high fluoride area and the Falkland Islands, about whose water supply no information was available. All determinations were made using the modified Willard and Winter chemical method.

TABLE I

## FLUORINE CONTENT OF WHOLE TEETH

| No. | Age | Sex | District         | Tooth Analyzed                                               | Degree of Caries | Per Cent Fluorine |
|-----|-----|-----|------------------|--------------------------------------------------------------|------------------|-------------------|
| 1   | -   | -   | Falkland Islands | permanent upper central incisors; pooled sample of two teeth | none             | 0.052(9)          |
| 2   | -   | -   | Falkland Islands | permanent upper central incisors; pooled sample of two teeth | moderate         | 0.050(2)          |



TABLE I (Cont'd)

| No. | Age                                    | Sex | District        | Tooth Analyzed                              | Degree of Caries                                                                         | Per Cent Fluorine |
|-----|----------------------------------------|-----|-----------------|---------------------------------------------|------------------------------------------------------------------------------------------|-------------------|
| 3   | Child -<br>age and<br>sex un-<br>known |     | Ripley,<br>Ont. | deciduous molar,<br>crown only              | none                                                                                     | 0.012(0)          |
| 4   | 15                                     | M   | Ripley,<br>Ont. | permanent molar                             | moderate caries<br>but exhibits<br>mottling as did<br>other teeth in<br>mouth of patient | 0.071(4)          |
| 5   | 11                                     | F   | Stratford       | deciduous second molar<br>L.R. (crown only) | slight                                                                                   | 0.013(2)          |
| 6   | 7½                                     | F   | Stratford       | permanent first molar<br>U.R.               | showed slight<br>decalcification                                                         | 0.031(4)          |
| 7   | 18                                     | M   | Stratford       | permanent first molar                       | severe, but tooth<br>showed mottling                                                     | 0.054(6)          |
| 8   | 22                                     | F   | Stratford       | permanent second molar                      | slight                                                                                   | 0.029(4)          |
| 9   | 42                                     | F   | Stratford       | pooled sample of 2<br>permanent bicuspid    | none                                                                                     | 0.039(8)          |
| 10* | 42                                     | F   | Stratford       | permanent second bi-<br>cuspid              | none                                                                                     | 0.049(2)          |
| 11* | 42                                     | F   | Stratford       | lower central incisor                       | none                                                                                     | 0.057(0)          |
| 12  | 10                                     | -   | Kitchener       | deciduous second molar<br>U.R.              | severe                                                                                   | 0.0075            |
| 13  | 14                                     | M   | Kitchener       | permanent first bicus-<br>pid               | none but showed<br>hyperplasia                                                           | 0.013(9)          |
| 14  | 15                                     | F   | Kitchener       | permanent second molar                      | moderate                                                                                 | 0.0083            |
| 15  | 19                                     | -   | Kitchener       | permanent second molar<br>L.L.              | moderate                                                                                 | 0.0082            |
| 16  | 21                                     | F   | Kitchener       | permanent third molar                       | none; tooth not<br>erupted                                                               | 0.0075            |
| 17  | 25                                     | M   | Kitchener       | permanent first molar<br>L.R.               | moderate                                                                                 | 0.024(7)          |
| 18  | 33                                     | F   | Kitchener       | permanent second molar<br>L.R.              | severe                                                                                   | 0.020(5)          |

\*

- Teeth No. 10 and No. 11 from same patient.



TABLE I (Concluded)

| No. | Age | Sex | District  | Tooth Analyzed                              | Degree of Caries | Per Cent Fluorine |
|-----|-----|-----|-----------|---------------------------------------------|------------------|-------------------|
| 19  | 39  | M   | Kitchener | permanent second molar                      | slight           | 0.033(1)          |
| 20  | 42  | F   | Kitchener | pooled sample of two lower central incisors | none             | 0.023(2)          |

Any comparison of sound and carious teeth made between different individuals for fluorine differences will likely introduce errors due to variation in the individual's fluorine exposure. Values listed in Table I show no significant difference in fluorine content between sound and carious teeth.

There appears to be a correlation between the fluoride content of the water and the percentage fluorine found in the teeth. Not enough results have been obtained for statistical analysis, but in general Stratford teeth have a higher fluorine content than Kitchener teeth. These results are in line with the findings of the previous survey by Miss M. Ham with respect to milk. (see DR 14 reports).

The increase in fluorine content of the teeth with age and exposure to dietary fluorides is also shown. The lowest values in Table I are for deciduous teeth and a third molar which had not erupted. Allowing for variation in the individual's fluorine exposure, there is a gradual increase in the fluorine content of permanent teeth with age. Tooth No. 11(Stratford) had the highest value of any of the Kitchener or Stratford teeth analyzed and was from a 42 year old patient. Tooth No. 7 (Stratford) had a value almost as high although it was from a much younger subject. This tooth also showed slight mottling of the enamel.

Tooth No. 4(Ripley) had the highest fluorine content in the table. Although the tooth was carious, it exhibited mottling as did all of the teeth in the patient's mouth.

The acquisition of fluorine through topical applications to the teeth of young dogs has recently been investigated in the Faculty of Dentistry of this University. The primary purpose was to see what effect fluorine treatments had on surface micro-hardness of the teeth, but fluorine analyses of the teeth were also made in this laboratory. Results of fluorine analyses on teeth from two of the dogs showed an increase of about 200 per cent in the treated teeth over the controls. See Table II.



TABLE II

FLUORINE CONTENT OF WHOLE DOGS' TEETH

| Control<br>%F |                                          | Teeth treated with 2% NaF<br>(8 applications)<br>% F |                                          |
|---------------|------------------------------------------|------------------------------------------------------|------------------------------------------|
| Dog 1         | 0.032 %<br>0.037 %<br>0.037 %            |                                                      | 0.087 %<br>0.101 %                       |
| Dog 2         | 0.034 %<br>0.029 %<br>0.029 %<br>0.028 % |                                                      | 0.103 %<br>0.103 %<br>0.127 %<br>0.098 % |
| Ave.          | 0.032 %                                  |                                                      | 0.099 %                                  |

Analysis of Enamel and Dentin Fractions of Teeth  
Extracted from Brantford Children

A number of the teeth which were sent to this department by Dr. H.K. Brown, chief of the Dental Health Division, Ottawa, have been separated into enamel and dentin fractions by the Manly and Hodge method. This separation has been greatly facilitated by the use of an electric grinding mill, recently acquired by the department, for pulverizing the tooth samples.

Both fractions have been analyzed for their fluorine content using the chemical method. The values obtained to date are given in Table III below.

TABLE III

FLUORINE IN ENAMEL AND DENTIN OF BRANTFORD TEETH

| Name            | Age | Tooth Analyzed                             | Degree of Caries | Enamel % Fluorine | Dentin % Fluorine | Ratio of Dentin to Enamel |
|-----------------|-----|--------------------------------------------|------------------|-------------------|-------------------|---------------------------|
| Melvin Freedman | 6   | deciduous lower molar                      | severe           | .0067             | .0093             | 1.4                       |
| Melvin Freedman | 6   | pooled sample of 2 deciduous second molars | moderate         | .010(2)           | .018(8)           | 1.8                       |



TABLE III (Concluded)

FLUORINE IN ENAMEL AND DENTIN OF BRANTFORD TEETH

| Name              | Age | Tooth Analyzed                       | Degrees of Caries | Enamel % Fluorine | Dentin % Fluorine | Ratio of Dentin to Enamel |
|-------------------|-----|--------------------------------------|-------------------|-------------------|-------------------|---------------------------|
| Salma Gassee      | 7   | pooled sample of 2 deciduous cuspids | none              | .0018             | .0046             | 2.6                       |
| Thelma Minshall   | 9   | deciduous molar (crown only)         | moderate          | .0085             | .012(0)           | 1.4                       |
| Gerald Owens      | 10  | deciduous lower first molar          | none              | .015(4)           | .014(3)           | 0.9                       |
| Robert Wm. Arthur | 5   | deciduous lower molar                | none              | .022(2)           | .020(2)           | 0.9                       |
| Marilyn Joyce     | 10  | permanent lower first molar          | moderate          | .032(4)           | .035(0)           | 1.1                       |
| Pearl Merridew    | 12  | permanent first molar U.R.           | severe            | .0049             | .011(6)           | 2.4                       |
| June Watson       | 12  | permanent upper first bicuspid       | none              | .0078             | .010(5)           | 1.4                       |
| James Low         | 12  | permanent lower bicuspid             | none              | .0074             | .022(8)           | 3.1                       |
| John Marsh        | 13  | permanent lower first molar          | severe            | .0032             | .016(7)           | 5.2                       |
| John Marsh        | 13  | permanent upper first molar          | severe            | .0062             | .012(4)           | 2.0                       |
| Annette Davidson  | 15  | permanent upper first molar          | severe            | .0029             | .011(4)           | 3.9                       |
| Joseph Hayward    | 15  | permanent upper first molar          | severe            | .0036             | .013(6)           | 3.8                       |
| Donald Whyte      | 17  | permanent lower first bicuspid       | severe            | .012(3)           | .022(1)           | 1.8                       |



Although large groups of teeth have not been examined, the ratio of fluorine in dentin to fluorine in enamel has a mean of 1.5 for deciduous teeth and a mean ratio of 2.7 for permanent teeth. (If the high value of 5.2 is not taken into account, the mean is 2.4 for permanent teeth.) McClure (2) refers to the relatively consistent ratio of fluorine in dentin to fluorine in enamel. His analyses on over 500 teeth indicate that dentin seems to contain about 2.0 to 2.5 times as much fluorine as enamel.

From the results above it would appear that there is a tendency for the fluorine content of the dentin to increase in relation to the fluorine of the enamel in permanent teeth.

### Application of the Microbiological Technique of Fluorine Analysis

Since it was hoped to do fluorine balance studies in this department, an attempt was made to apply the microbiological technique of fluorine analysis outlined by L.L. Winter to samples of urine.

In urine, the high chloride content necessitates the addition of large amounts of silver sulphate when analyzed by the chemical method. The silver sulphate tends to cause violent bumping in the distillation flask, and it was felt that it would be advantageous if the microbiological method could be used. Duplicate samples of urine analyzed by the chemical method gave .57 and .59 p.p.m. of fluorine (ave. .58 p.p.m.). The daily output of fluorine would be 319<sub>y</sub> or .319 mg.

The microbiological technique was tried using a 24 hour sample from the same subject. To part of the sample fluorine amounting to 6 p.p.m. was added to test recovery. The equivalent of .396 mg. of fluorine was found for the 24 hour period. The urine plus 6 p.p.m. showed only 4.9 p.p.m. in the recovery test. Nevertheless, the method was felt to show promise for analyzing urine.

In a paper by Loevenhart and Pierce, 1907, (3) it had been stated that the enzyme lipase was inhibited by fluorine. Since a method for testing the action of various adjuncts on lipase was in use in the department, it was thought advisable to test the response of the enzyme to graded increments of fluorine. It was felt that the pure enzyme might be more sensitive to fluorine than the microorganism *L. acidophilus* and that a method of fluorine analysis might be worked out using the enzyme. The substrate for the lipase is "Tween 20", an organic surface active agent which is a non-ionic ester-ether derived from hexahydric alcohols, alkylene oxide and fatty acids. The action of the enzyme is determined by titrating acid produced by hydrolysis of substrate.



In preliminary trials with this enzyme method it appeared to be no improvement on the microbiological. An economic comparison was made of these two procedures and the cost of the materials per determination of the enzyme method was about 10 times that of the microbiological one. However, the time to perform each would amount to about the same.

March, 1950

Subject: Measurement of Physiological Forces in the Mouth

Investigator: Robert E. REFERENCES

Project: 25-29

- (1) Box, H.K., and Hodgins, H.J., Water and Sewage, May, 1944.
- (2) McClure, F.J., J. Dent. Res., 27:287, 1948.
- (3) Loevenhart, A.S., and Pierce, G., J.B.C., 2:397, 1907.

(a) The measurement of the strength of bite of the jaw is a problem which has to be approached by the study of the dentition and face.

(b) The measurement of the extent of use of the jaw and physiological in operated and non-operated cleft palate individuals.

(SIGNED:) M. Doreen Smith

(c) The measurement of the forces involved in the chewing of food is a problem which has to be approached by the study of the dentition and face.

Inasmuch as the apparatus ordered under this grant have only recently arrived in Toronto, even preliminary findings are as yet not reportable. It should be noted, however, that the various methods of adapting the device to the measurement of physiological forces during the chewing of food are being indicated in providing us with an accurate procedure to date.





APPENDIX "O"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: Measurement of Physiologic Forces in the Mouth  
Grantee: Robert E. Moyers  
Project: DR 26

SUMMARY OF WORK

This project has been broken down into three separate studies:

- (a) The measurement of the strength of some of the sucking habits said to be detrimental to the growth of the dentition and face.
- (b) The measurement of the extent of use of the palatal and pharyngeal in operated and non-operated cleft palate individuals
- (c) The measurement of the forces inherent in the standard orthodontic appliances used for tooth alignment.

Inasmuch as the apparatus ordered under this grant have only recently arrived in Toronto, even preliminary findings are as yet not reportable. It should be said, however, that the various methods of adapting the strain gauge to the measurement of physiologic forces within the mouth showed every indication of providing us with our most accurate procedure to date.

/c

Robert E. Moyers





REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Subject: Measurement of Physiologic Forces in the Mouth  
Grantee: Robert E. Moyers  
Project: DR 26

Only within the last month have the apparatus ordered with the funds of this grant arrived in Toronto. They are now being assembled and the research is being undertaken. The project has naturally broken down into three separate studies, viz. (a) The measurement of the strength of some of the sucking habits said to be detrimental to the growth of the dentition and face. (b) The measurement of the extent of use of the palatal and pharyngeal muscles in operated and non-operated cleft palate individuals. (c) The measurement of the forces inherent in the standard orthodontic appliances used for aligning the teeth. In none of the three studies can even a preliminary set of values be reported at this time. It can be said however that the suggested method of measuring is proving to be a most accurate method of making the determinations. The methods of adapting the strain gauges to the various studies are as follows.

(a) Sucking habits: These are being studied by means of strain gauges within hydrostatic systems (physiologic pressure transducers). The transducer is connected by means of rubber tubing of small bore and thick walls (ureteral catheters) to a latex bulb which is introduced into the mouth. In the case of thumb sucking the latex bulb is attached to the thumb of a surgeon's rubber glove. This brings the small bulb into a position between the thumb and the palate where variations in force application are immediately recorded. By means of various sizes and shapes of these 'blisters' or bulbs we are studying thumb and finger sucking pressures, lip sucking pressures, tongue sucking, and other muscular pressures claimed to be detrimental.

(b) Cleft palate muscle study: By using the above mentioned hydro-statically arranged strain gauge system we are studying the use of these muscles in cleft palate individuals. An impression of the cleft is taken, a stone model poured and the latex bulb made to exactly conform to the shape of the cleft. These findings should provide a clue as to which surgical procedures are more efficacious as far as cleft palatal

muscle usage is concerned and which types of obturators provide the most efficient closure of naso- from the oral-pharynx during speaking and deglutition of food.

(c) Orthodontic appliance force: An ordinary strain gauge has been fitted with several different adaptors to allow its use against the various types of standard orthodontic mechanism. Direct readings are thus possible of being recorded.

Robert E. Moyers



APPENDIX "P"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: Development of a photoelectric colorimetric method for determining small amounts of fluorides.

Grantee: O. J. Walker

Project: DR 28 Amount of Grant: \$1,000

SUMMARY OF WORK

A Photoelectric Colorimetric method has been developed for determining small amounts of fluorides in natural waters. The method is based on the bleaching by fluorides of the red lake formed by a zirconyl salt and sodium alizarin sulfonate. The method is rapid and of good precision. If the sulfate content is over 150 p.p.m. there is interference. Means for correcting for this ion have been worked out. Phosphate ions do not interfere if their concentration is less than 2 p.p.m. In higher concentrations the behaviour is erratic and means to correct for these are being studied. For example, an investigation is under way to learn if phosphate ions can be tied up by borates. A study is under way for developing a more accurate photoelectric colorimetric method for determining phosphates.

The reaction involving the bleaching of the lake formed by aluminum salt and hematoxylin is being studied from the standpoint of developing another rapid photoelectric colorimetric method for fluorides.





## APPENDIX "P"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: A photoelectric method for the determination of small amounts of fluorine

Grantee: O.J. Walker

Project: DR 28 Amount of Grant: \$1,000

Progress has been made on the development of a photoelectric colorimetric method for the determination of small amounts of fluoride in waters. The method is based on the bleaching by fluoride of the lake formed from a zirconyl salt and sodium alizarin sulfonate. The procedure followed in preparing the lake is the Scott<sup>(3)</sup> modification of the Sanchis<sup>(2)</sup> method for the colorimetric determination.

The absorption spectrum of the lake was determined in a Hilger-Nutting Spectrophotometer. See Figure 1, Curve A. Maximum absorption is such to be in the region of 500 m $\mu$ . It was also necessary to determine the absorption spectrum for the dye, sodium alizarin sulfonate and this is Curve B in Figure 1. It will be seen that maximum absorption is small in the 520 m $\mu$  region but quite considerably farther in the ultra violet.

In the photoelectric colorimetric method, use was made of the Lumetron photoelectric colorimeter. The color filter chosen was one that cuts out all light except that with wave lengths in the region of 520 m $\mu$ . To obtain more accurate readings an absorption cell, 15 cm. in length, was used.

The colorimeter was calibrated in this manner: Distilled water was placed in the absorption cell and the instrument adjusted to read 100 on the slide wire. In the meantime solutions containing known amounts of fluoride were treated with the same amounts of reagents and given the same periods of aging as specified in the Scott colorimetric method. These were placed one by one in the sample holder and the slide wire reading obtained. Based on samples of water of 100 ml., a curve in which parts of fluoride per million parts of water were plotted against slide wire readings was constructed. This calibration curve is shown in Fig. 2. It will be noted that this curve is not a straight line as it would be if Beer's Law were obeyed. This deviation from Beer's Law is due to the fact that one is dealing with two colored substances, one of which is absorbed strongly at the wave length involved and the other only slightly. (See Figure 1.)



As is true of the corresponding visual method, this method is good for amounts of fluoride from 0 to 1.4 p.p.m. based on a 100 ml. sample.

This calibration curve can be used in the analysis of waters containing unknown amounts of  $F^-$  as long as the fluoride content is less than 1.4 p.p.m. With larger amounts of fluoride, smaller samples are taken and diluted to 100 ml. with distilled water. The method is quite rapid and fifteen or twenty samples can be analyzed easily in a day. As can be seen from the curve, the method is accurate to better than 0.1 p.p.m. of fluorine.

Quite a bit of attention has been given to the reproducibility of results. It has been found that a great deal of care has to be taken that the same amounts of reagents be used each time. Slight changes in the amounts of zirconyl salt and sodium alizarin sulfonate added have quite an effect on the slide wire readings. In this method, one makes up a solution containing these two reagents and thus these are added together. As long as the same volume of this solution is used the values obtained agree with the calibration curve. However, when a new solution of these is prepared and used, it has been found necessary to prepare a new calibration curve.

### Effect of Interfering Substances

#### 1. Sulfate Ions:

The effect of sulfate ions was determined by using samples of water containing known amounts of sulfates and fluoride. If the sulfate content was less than 150 p.p.m. the fluoride content found was the same as the fluoride added. With larger amounts of sulfate there was some interference. The interference was of the same order as found by Walker and Finlay<sup>(5)</sup> for the visual method. The effect of sulfates has been found to be additive so if one knows the concentration of sulfates in the water, it is an easy matter to correct for them.

#### 2. Phosphate Ions:

It has been known that phosphates interfere seriously with the visual zirconyl salt - alizarin sulfonate method for determining fluorides. In our experiments it has been possible to show that if the phosphate content of the water is from 0 - 2 p.p.m. there is no interference. However, with higher concentrations of phosphates, the results obtained are erratic. When the water contained about 10 p.p.m. of phosphate and amounts of fluoride from 0 - 0.6 p.p.m. results for fluoride were high but if the fluoride content were from 0.7 to 1.5 p.p.m. the results for fluoride were low.



For most natural waters, the phosphate content is seldom over 2 p.p.m. so for these the presence of phosphates is no problem. However, for the exceptional water and also if this method for determining fluorine is to be extended to plants, bones and teeth some modification of the method should be developed in which corrections can be applied for the phosphate present.

We are now investigating agents that may be able to tie up phosphates in stable complexes but will at the same time not interfere with the bleaching action of fluoride on the lake. Borates are a possibility and their effects are now being investigated. It has been found that borates up to 140 p.p.m. have no effect on the action of fluorides. Results have not yet been obtained as to whether borates will tie up phosphates. This phase of the investigation is proceeding.

A method for the rapid determination of small amounts of phosphates is of prime importance in this research. Colorimetric methods involving the formation of molybdenum blue have been studied. The one proposed by Stoloff<sup>(4)</sup> may be adapted to photoelectric colorimetry. The determination may be carried out rapidly with good precision. In our experiments in which the 15 cm. absorption tube is used the sensitivity is about seven times that claimed by the author of this method. However, there are several difficulties in the determination that are still to be ironed out.

### Other Studies

A beginning has been made on the development of a second photoelectric method for the determination of fluorides. It is based on the decolorization of a solution containing aluminum salt and hematoxylin by fluorides. This reaction was first studied by Okuno<sup>(1)</sup> and served as a basis of a visual method for fluorides. We are especially interested in this method as ions such as sulfates and phosphates may not interfere in the same way as in the zirconyl salt, alizarin sulfonate method.

The experimental work is being carried out by Mr. J.H. Martin and it will be used in partial fulfillment of the requirements of a M.Sc. degree at this University.

### Bibliography

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EXTINCTION  
COEFFICIENT

ABSORPTION SPECTRA OF  
SODIUM ALIZARIN SULFONATE  
AND OF THE BELLANE



Figure 1







SLIDE WIRE  
READING

CONVERSION OF SLIDEWIRE  
READINGS TO P.P.M. F<sup>-</sup>ION  
SANCHIS METHOD (MODIFIED)

80.0

75.0

70.0

65.0

60.0

55.0

50.0

45.0

40.0

0.2

0.4

0.6

0.8

1.0

1.2

1.4

P.P.M. F<sup>-</sup>

Figure 2







## APPENDIX "Q"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches.

Grantee: Norma Ford Walker

Project: DR 15

#### SUMMARY OF WORK

(This is a brief statement from a longer report submitted to the Grantee.)

In the report submitted to the committee in September 1949, it was pointed out that the eruptive times of the teeth as well as measurements of the widths of the dental arches are basic to this study. The past five months have been spent in analysing the results obtained from these basic calculations.

#### Analysis of the eruptive time of the teeth

- a) Comparing the eruptive pattern of a random group of girls with a random group of boys.
- b) Comparisons made within each sample.
- c) A comparison of the parts played by heredity and environment.

The mean eruptive time ( $\bar{X}$ ) in days, the standard deviation ( $S$ ), the standard error ( $S_x$ ) and standard error of the difference ( $S_{diff}$ ), the sexes separated constituted the first calculations. From these statistics, it is possible to show that no true difference exists in the eruptive time of the deciduous teeth of a random group of boys and girls (although published statements to the contrary have been made).

From further statistics it has been observed that:

1. No true difference exists between the means of the eruptive time for twins and singletons in each of the sex categories (although the prenatal environment of a twin birth is known to be more unfavourable than that of a single birth).

(SUMMARY)

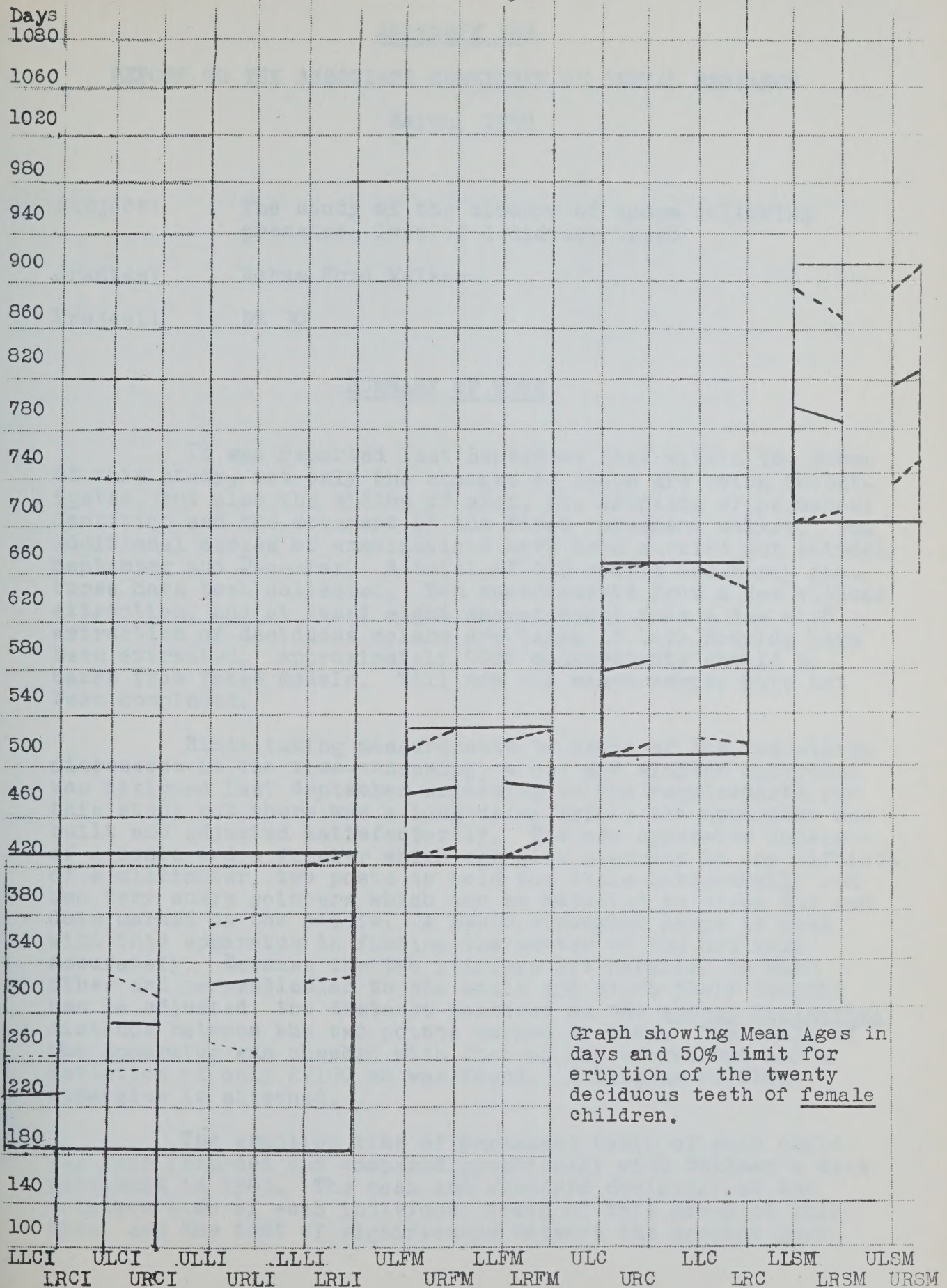
2. The similar prenatal environment of fraternal twins appears to be a factor of little significance in its effect on the eruptive pattern.
3. Random groups with different hereditary factors show a far greater difference in eruptive times than groups with the same hereditary factors.

As a result of the foregoing analyses, final graphs have been made to show the eruptive pattern for each sex. The series of eight homologous teeth have been marked off into four blocks, to emphasize the breaks which occur between the first four, the second two, the third two, and the fourth two pairs.

Analyses are now underway comparing the type of occlusion with the eruptive pattern for each individual studied. To date 81 individuals have been analysed in an attempt to associate the eruptive pattern with irregularities from normal occlusion.



# APPENDIX "Q"







## APPENDIX "R"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1950

Subject: The study of the closure of space following premature loss of deciduous teeth

Grantee: Norma Ford Walker

Project: DR 30

#### SUMMARY OF WORK

It was reported last September that within the scope of this study, not only the changes of space are being investigated, but also the widths of arch, the eruption of permanent dentition and the movement of the first permanent molars. Two additional series of examinations have been carried out between September and December. A total of 610 models and x-ray pictures have been collected. Ten measurements from a jaw without extraction, and at least eight measurements from a jaw with extraction of deciduous molars are taken if both cuspids have been extracted. Approximately 5000 measurements should be taken from these models. Till now the measurements have not been completed.

Since taking measurements by means of the travelling microscope is too time-consuming, a new and simpler apparatus was designed last September according to the requirements for this study but there was a long delay before the apparatus was built and adjusted satisfactorily. The new apparatus consists of a scale and a vernier which can give accuracy to one fiftieth of a millimeter, two posts to hold the scale horizontally and two very sharp pointers which can be adjusted to touch the red dots marked on the models. A Beebe Binocular Loupe is used with this apparatus in finding the center of the dot more accurately. Because the two pointers are parallel to each other and perpendicular to the scale and since their lengths can be adjusted, the distance measured is the actual horizontal distance between the two points marked by dots. The scale of the apparatus was checked with that of the microscope and a deviation of only 2/100 mm was found. A diagram of this apparatus is attached.

The eruption time of permanent teeth of each child has been recorded and compared graphically with Hellman's data published in 1943. The mean and standard deviation of the eruption time of each individual tooth of this group of children, and the test of significance between the present data



(SUMMARY)

and Hellman's will be calculated later when the data is completed.

The widths of arch taken from the models of each case have been compared graphically with Cohen's data to find the difference between the individual case and Cohen's average published in 1940. The mean width of the arch, the standard deviation and the test of significance between these two groups will also be worked out when the data is completed.

From studies of the measurements of changes of space, it has been found that the amount, rate, and the type of changes of spaces vary from one case to another, even among children of nearly the same age, having the same extractions and living under similar familial conditions. It seems that each case follows its own pattern. This makes the writer think that if the premature loss of deciduous teeth is a cause of malocclusion, it may have certain hereditary factors which control the loss of space. The writer believes that if we could observe premature loss of deciduous teeth in twins, we might determine to what extent heredity plays a role in the closure of space and malposition of permanent successors.



## APPENDIX "S"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: Experimental Artificial Production of Dental Caries  
in Vitro.

Grantee: H. K. Box, D.D.S., Ph.D. and R.A. Hugill, D.D.S., B.Sc.  
(Dent.)

Project: DR 18 Amount of Grant: \$1620.

#### SUMMARY OF WORK

This project was undertaken with the hopes of producing an artificial caries lesion in vitro, free of bacterial activity, that would closely resemble many of the characteristics of the pathological caries lesion. That is to say, an attempt would be made to employ chemical compounds that would imitate the destructive metabolic products of the organisms associated with the carious process. In this manner a controlled experiment would be set up. Following this, after presumably achieving success in the first step in initiating artificial caries, various theoretical protectives might be sought out and tested against a controlled experiment. Such is the unlimited scope of this project.

##### (a) Enzyme Experiments

The three enzymes used were pepsin, papain, and trypsin, as well as pepsin and papain in conjunction. Results showed surface (also slight penetration) pigmentation and minute loss of inter-prismatic substance with the exception of trypsin action. Papain and pepsin when used together produced more profound results.

##### (b) Acid Experiments

Next, experiments with lactic acid were carried out to determine further effects of acids if possible. Results were identical as those described previously, that is, cross-striation of the enamel rods in early decalcification to complete decalcification with loss of structure in the final stages.

##### (c) Acid-Enzyme Experiments

To follow up these individual experiments with acids and different enzymes, both "factors" were used simultaneously to set up the artificial "caries mechanism" that has been described in earlier reports. In explanation of the set up, "it" consists of a combined solution of acid (lactic acid principally used) along with proteolytic enzymes (pepsin and papain). It will be recalled that many of the significant characteristics of the natural caries lesion were duplicated artificially through



this means. It is significant to note that when papain was used in conjunction with lactic acid or as a follow-up solution to one combining lactic acid and pepsin enzyme that the pigmentation and organic disintegration or digestion was somewhat more pronounced. This might be expected since papain is capable of breaking down proteins further than pepsin.

#### (d) Caries Hypothesis

An important phase of the caries process involves an enzymatic hydrolysis of enamel and dentin matrix. There are probably at least four type enzymes participating simultaneously.

Sulphatase - which releases the protein radical from the sulphated complex chondroitin sulphate molecule of enamel and dentin.

Proteases - further the breakdown of the protein molecule of enamel and dentin.

Tyrosinase enzyme which presumably assists in the pigment formation of caries.

Hyaluronidases which may assist in the breakdown of connective tissue ground substance (dentin).

Although enzymatic action may play a key role in the process, organic, and to some extent inorganic acids liberated through bacterial metabolism and other means, play a vital and intricate part in the same caries process. They are responsible for the dissolution of inorganic carbonates, sulphates, phosphates, etc. of enamel and dentin matrix. They also probably tend to act as catalysts for enzymatic activity, and at the same time limiting the field of enzymatic hydrolysis to those group of enzymes which are capable of activity in an acid pH. medium. It is, therefore, unlikely that alkaline enzymes such as pancreatic trypsin take part in the enzyme hydrolysis of tooth tissues.

#### (e) Experiments to Demonstrate and Investigate Caries Protective Agents

Assuming that a caries mechanism has been to some degree successfully established, a series of experiments are being done to test out theoretical protective agents against the artificial caries-producing solutions. Among the chemical solutions being used are: Oleic acid, Linoleic acid, ricinoleic acid, nucleic acid, sodium fluoride, calcium fluoride, fluorsal, casein, bone meal, calcium glycerophosphate, sodium benzoate, sodium salicylate, sodium oleate, ammoniated dentifrices.



Experiment 1. Teeth were emersed in solutions of lactic acid and proleolytic (pepsin and papain) enzymes to which was added one of the above mentioned list of chemicals. Results after three months showed no decalcification or proteolysis on the surface of tooth whatsoever.

Experiment 2. Some of the teeth used in experiment number 1, were removed from the solutions and placed in 5% solution of lactic acid. Results in 24 hours showed signs of acid decalcification on each tooth surface.

Experiment 3. An attempt was made to neutralize the protective effect of the fatty acids used namely-oleic, linoleic and ricinoleic, by adding to the compound solution some fat splitting lipase enzyme. Results were negative, ie. no changes took place on the surface of the enamel.

### Conclusions

- (a) Various chemicals are capable of producing a film on the tooth surface which resists acid and proteolytic enzyme attack.
- (b) This film persists as a protection against acid and proteolytic enzyme attack only when the chemical agent producing the film is constantly present in the solutions of acid and enzyme.
- (c) Fat splitting enzymes do not seem to be capable of removing or neutralizing the fatty-acid films placed on the teeth if the fatty acid chemicals producing the film remain constantly in the solution.
- (d) A hypothesis on oral salivary protection might be evolved from these results:
  - (i) certain materials present in the saliva of caries immune mouths may be capable of laying down a film on the teeth which is resistant to acid and enzyme attack.
  - (ii) these same materials may be absent in the saliva of caries susceptible mouths.
  - (iii) the characteristic acid pH. of the saliva would favour the precipitation of these materials out of the saliva onto the teeth.

To prove or disprove this hypothesis a few experiments should be carried out. An outline of proposed experiments will be made at the forthcoming Annual Meeting.





## APPENDIX "S"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1950

Subject: Experimental artificial production of dental caries in vitro.

Grantee: H.K. Box, D.D.S., Ph.D., and R.A. Hugill, D.D.S.,  
B.Sc.(Dent.)

Project No.: DR 18                      Amount of Grant: \$1620.

### ANNUAL REPORT

Purpose: In the past, experimental caries have been produced in vitro i.e. on extracted teeth. However, many of these research undertakings could not be classified as "controlled experiments" due to the uncertainty and variances of the activity of the organisms present that are presumed to have been the activating agents in the caries process.

Recently, a great deal of effort has been made in search of some protective agent to inhibit the carious process in teeth. For example, in the use of fluorine as a protector, it is undergoing exhaustive research in an attempt to ascertain its value. Presumably, in the main, if stated in a simple way, it is thought to render the surface enamel more resistant to acid attack.

However, it still remains to be said that if we are to find some means of protection we must first understand reasonably well the chemical processes involved in dental caries.

With these thoughts in mind this project was undertaken with the hopes of producing an artificial caries in vitro free of bacterial activity that would closely resemble many of the characteristics of the pathological caries lesion. That is to say, an attempt would be made to employ chemical compounds that would imitate the destructive metabolic products of the organisms associated with the carious process. In this manner a controlled experiment would be set up. Following this, after presumably achieving success in the first step in initiating artificial caries, various theoretical protectives might be sought out and tested against a controlled experiment. Such is the unlimited scope of this project.

Scope: All technique was carried out in vitro, mostly on extracted human teeth. Some experimental work was done on extracted teeth of hogs and cattle. All teeth were carefully selected, free from dental caries upon visual examination. Following mechanical prophylaxis they were coated in entirety with either parafin wax or black compound, leaving uncovered only a small surface or "window space" on the labial surface of each





## "S-2"

individual tooth. Thymol crystals were placed in all the experimental solutions used, in order to inhibit bacterial activity. All sections made of the teeth were calcified ground sections.

First, the action and the resultant effects of acids upon tooth enamel and dentin were investigated. Several different acids, both organic and inorganic varying in concentration, i.e. percent solution, and in pH. were used in the technique. The acids initially used were the following organic acids, - lactic, citric, tartaric, succinic, malic, propionic, butyric, formic, pyruvic, and the following inorganic acids, phosphoric, acetic, hydrochloric, nitric. Approximately 1% solutions pH. 4.5 were used.

The rapidity of the changes produced in the tooth structures varied with the specific acid used. All the acids used produce similar effects but in varied degrees of intensity.

Acid action appears to be responsible for the following changes in tooth structure: first in the enamel:

- (a) enamel becomes chalky and opaque on macroscopic examination
- (b) cross-striations of the enamel prisms appear or become more pronounced.
- (c) destruction of the interprismatic spaces is apparent which become more translucent and more apparent.

Second - the changes produced in dentin:

- (a) a general translucent homogeneous area immediately below the area of enamel decalcification, that is in that area nearest to the surface of the tooth, is apparent.
- (b) there is a general loss of dentin structure immediately next to the dento-enamel junction below the calcified enamel in that the dentinal tubules appear less distinctive or to some degree obliterated from view.
- (c) beyond the translucent zone a dark opaque zone is formed, not unlike that of metamorphosed dentin in appearance.
- (d) there is an area of dentin shrinkage at the dento-enamel junction beneath the calcified enamel zone.
- (e) interglobular spaces appear to be much less indefinite in structure, i.e. they appear to be more homogeneous with the surrounding dentin structure.





However, there is one point which is most important to note here, that acid decalcification produces no pigmentation characteristic of caries and fails to account for many characteristic phases and appearances of the dental caries lesion.

The next step in the project was to investigate the changes that might be produced by enzyme action, especially on the protein organic content of enamel and dentin.

Several enzymes were used, namely, pepsin, papain, trypsin, ptyalin, amylase. The pepsin and papain enzymes are active in acid pH. ranges and the solutions used were approximately pH. 4.5 whereas the other enzymes namely, trypsin, ptyalin and amylase become activated in an alkaline pH. range.

After the teeth were subjected to the action of these enzymes for a period of about five months the following observations could be made -

firstly, the enzymes that are active in an alkaline pH. range produced no effect on the enamel of the teeth.

secondly, those enzymes namely pepsin and papain which are active in an acid pH. range produce some changes in enamel which can be stated thusly.

- (a) Microscopically there was a brownish pigmentation of the enamel rods and the interprismatic substance in the central area of the affected zone.
- (b) On the margins of the same zone the action of the enzyme appears more diffuse, showing partial digestion and pigmentation of the interprismatic substance whereas the rods remain relatively unaffected.
- (c) In regions where only the interprismatic substance was pigmented orangey-brown, a lighter pigmented "fuzzy" appearance seemed to penetrate towards the core of the enamel prism from the interprismatic substance. This might suggest the beginning of the proteolytic attack on the enamel prism itself.
- (d) On the advancing border of the affected zone the interprismatic substance appears to be attacked first, showing orangey-brown pigmentation.
- (e) The enamel rods showed marked cross-striation because of the prolonged slow decalcifying action of the weak acid pH. and acid concentration of the enzyme solution.





In summarizing we have reason to believe that -

- (a) enzymes attack the organic content of the interprismatic substance first, digesting it and then producing an orangey-brown pigmentation.
- (b) enzymes next appear to attack the more resistant organic complex substances of the enamel rod itself by a "lateral attack", rather than down through the centre of the rod.

To follow up these individual experiments with different acids and different enzymes, both "factors" were used simultaneously to set up the artificial "caries mechanism". In explanation of the set up it consists of a combined solution of acid (lactic acid principally used), along with proteolytic enzymes (pepsin and papain). Many of the significant characteristics of the natural caries lesion were duplicated artificially in six months' time by this means. Many changes produced artificially characteristic of caries of the enamel are -

- (a) Pigmentation. In the sections in which the process is entirely in the enamel and had not reached the dento-enamel junction there was a darkly pigmented orangey-brown band in advance of the carious lesion. This band is more darkly pigmented than the pigmentation found in the enamel prisms. On the border of the pigmented band are little orangey-brown "icicles" projecting into the adjacent partially broken down matrix. Thus it appears that the decalcifying-proteolytic process, initiated by the lactic acid and pepsin enzyme, appears to work down between the rods or along the enamel prism sheathes in advance of the same process working down through the centre of each enamel prism.
- (b) Pigmentation of the enamel prisms. The pigment produced was a definite orangey-brown and was confined only to the area below the "window space".
- (c) Transverse striation. These are cross-striae seen on the enamel prisms and appear to be double lines.
- (d) Destruction of the interprismatic substance with minute spaces between the enamel rods. This may be due to swelling of the enamel sheathes, or a complete digestion of the interrod substance.
- (e) The zone lying beneath the "window space" presented both areas of translucent enamel and areas of granulated more opaque enamel substances.





"S-5"

The changes produced artificially characteristic of certain changes in the dentin in caries are the following:

- (a) A yellowish-orange pigmentation of the dentin below and at the dento-enamel junction.
- (b) Dentin shrinkage at the dento-enamel junction beneath the carious lesion of the enamel.
- (c) Translucent area beneath the zone of yellow pigmentation.
- (d) Opaque zone beyond the zone of translucent dentin.
- (e) Interglobular spaces appear more homogeneous with the surrounding dentin structure.

It is significant to note that when papain was used in conjunction with pepsin enzymes or as a follow up solution to the one combining lactic acid and pepsin enzyme that the pigmentation and organic disintegration or digestion was somewhat more pronounced. This might be expected since papain is capable of breaking down proteins further than pepsin.

The significant conclusions that can be drawn from the observations of the experiments on the phases of caries in human teeth are the following:

- (a) Various acids have been acclaimed by researchers in different parts of the world as the specific acid responsible for the acid decalcification phase in the dental caries process. Experimentation shows that many acids will produce decalcification of the enamel, but certain acids are definitely more active than others. It is quite probable that it is the relative pH. that is more important than the specificity of any particular acid in the acid phase of caries. The emphasis placed on the role of acid in the production of dental caries has undoubtedly served to obscure the broader and more fundamental mechanism concerned in the caries production. The evidence to date shows that the acid attack on enamel does not produce the total microscopical picture of enamel caries.
- (b) Proteolytic pepsin enzyme attacks the organic content of teeth and the changes produced characteristic of caries are more pronounced when activated by acid action. Pepsin breaks or digests the protein content of enamel and dentin down to a certain level whereas papain enzyme appears to carry the process of proteolysis even farther.





- (c) A combined acid enzyme attack produces many of the changes that are produced in a frontal caries process. Thus it seems evident that an artificial caries producing mechanism can be set up in a test tube without the aid or interference of bacteria; however, there are many features of the proteolytic phase of dental caries yet to clarify.

From these experiments, the results thus obtained strongly supports the theory that the carious process must comprise of an intermittent or simultaneous enzyme-acid reaction, in which acid and enzyme destroy or digest the inorganic and organic content respectively of enamel and dentin. Since the results of these experiments show that the effects of intermittent or simultaneous acid and enzyme attack are the essential phases of the carious process, it is obvious that they must chemically represent the constituent destructive metabolic products from the bacteria associated with the caries picture.

In the past, much emphasis has been placed upon the importance of decalcification (by acids) as the principal phase of the caries process, while the enzyme phase was rather under rated in importance. Since the knowledge of specific enzyme activity has so advanced in recent years, it is not incredible to believe that tooth enamel and dentin could be broken down a considerable extent by a series of bacterial enzymes in association with the acid decalcification process.

There are a few important facts found in recent literature that might be advantageously recorded here.

1. Pincus states that quantitative estimations of nitrogen and sulphur together with evidence from the developmental aspect and from histochemical tests suggest that enamel protein is a mucoprotein.
2. A substrate resembling chondroitin sulphuric acid by qualitative tests has been prepared from dentin (Pincus).
3. From this substrate (chondroitin sulphuric acid) enzymes of gram negative bacilli in pure culture have been capable of quantitative release of sulphatase.
4. Chondrosulphatase (a bacterial sulphatase) is capable of hydrolizing or releasing sulphate from chondroitin sulphuric acid.
5. Bacteria are also capable of releasing an enzyme tyrosinase which is capable of oxidizing tyrosin (a constituent of enamel and dentin protein) to a brownish black pigment called melanin.





6. Hyaluronidase enzyme often referred to as the "spreading factor" of infection, acts on and hydrolyses the ground substance of connective tissue. Certain bacteria are capable of liberating this enzyme when metabolizing on a suitable substrate.

Thus a different explanation or hypothesis of caries development in enamel and dentin may be as follows:

Enamel protein resembles a mucoprotein in properties. Gram-negative bacilli commonly found in caries can release sulphatase (possible chondrosulphatase). Enamel is made up of hexagonal prisms each surrounded by a sheath of enamel protein. Enzymatic hydrolysis of these sheathes in caries, with release of sulphuric acid, would result in attack on each prism from the periphery inwards: the relatively insoluble calcium phosphate which forms a considerable portion of the inorganic part of the enamel would be slowly changed to the relatively soluble calcium sulphate. It is of interest to notice that Frisbie shows enamel caries proceeding in just such a manner, each prism decreasing in size from the outer edge inwards. The pigmentation associated with dental caries in enamel and dentin has been shown to be an insoluble melanin type pigment accompanied by other soluble pigments. Bacterial tyrosinase quite likely plays an important role in the production of melanin pigment. Hyaluronidases also probably contribute to the caries process, assisting in the break-down of the ground substance of connective tissues, (dentin). Bacterial proteases and acid tryptases such as papain must assist in the organic protein digestion of enamel and dentin. Although enzymatic action may play a key role in the process, organic and to some extent possible inorganic acids, liberated through bacterial metabolism and other means, play a vital and intricate part intermittently or simultaneously in the same caries process. They are responsible for the dissolution of inorganic carbonates, sulphates, phosphates, etc. of enamel and dentin matrix. They also probably tend to act as catalysts for enzymatic activity and at the same time limiting the field of enzymatic hydrolysis to those group of enzymes which are capable of activity in an acid pH. medium. It is therefore unlikely that alkaline enzymes such as pancreatic trypsin take part in the enzyme hydrolysis of tooth tissues.





Such a hypothesis cannot be disregarded or turned aside without intensive investigation. It is the author's hope that such a project would be supported for the coming term.

References were made earlier to the fact, that if a caries producing mechanism could be set up, it might be used to seek and test out protective agents that might inhibit dental caries.

Assuming that a caries mechanism has been to some degree successfully established, a series of experiments are being done to test out theoretical protective agents against the artificial caries producing solutions.

The initial experiment was set up thusly:

1. Non-carious extracted human teeth were selected.
2. The teeth were mechanically cleaned and the entire surface of each tooth was isolated except for a small window space on the labial surface.
3. The so-coated teeth were placed in each of the following solutions (distilled water) -  
Oleic acid, linoleic acid, ricinoleic acid, sodium fluoride, calcium fluoride, fluorosal, calcium glycerophosphate, bone meal, nucleic acid, sodium salicylate, sodium benzoate, ammoniated dentifrices. Casein, sodium oleate.
4. Then the artificial caries producing mechanism was added to each solution, that is, a combined solution of lactic acid and pepsin and papain enzymes.
5. Thymol crystals were added to control and inhibit bacterial activity.

Observations made after three months show that there is no sign of acid decalcification or enzyme proteolysis upon the surface of the enamel. Theoretically these protectives seem to produce a film on the teeth which inhibits acid enzyme action.

To follow up this experiment some of the teeth that had been in the artificial "caries producing solution" with the "various protectives" added for a considerable length of time, were removed and placed in 5% lactic acid solution. In less than 24 hours all the teeth except for those that were placed in fluoride solutions showed evidence of the beginning of acid decalcification on the enamel surface. After two or three days, those





teeth that had been in the fluoride solutions began also to show evidence of decalcification. Further experimentation showed that decalcification could be produced much more readily on fluoride treated teeth if the teeth were either washed thoroughly with tap water for a few hours, or shaken up in an alkaline solution - sodium hydroxide - and then subjected to acid treatment.

Then experiment was set up to test whether fat splitting enzymes would have any effect on the effectiveness of fatty acids as protective agents. Into the solutions of lactic acid and proteolytic enzymes in which teeth had been suspended for three months, lipase enzyme (fat splitting enzyme) was added. Results showed that the lipase enzyme seemed to have no apparent effect upon the effectiveness of the fatty acids as protectives, that is, no changes occurred on the surface of the teeth.

There are a few conclusions that might be drawn from the above findings.

1. Various chemicals that are capable of producing a film on a tooth seemed to be able to inhibit acid decalcification and enzyme proteolysis. Applied physics may offer an explanation. The surface of a tooth theoretically has a negative charge and when in an acid solution positive charges leave the tooth surface and attract and unite with the negative radical charge of acids, consequently allowing the acid radical to reach the surface of the tooth and cause decalcification. However, the chemical protectives in the acid solutions placed a positive charged film on the surface of the tooth. Negative charges leaving the film surface repel the negative charges of the acid molecule and prevent the acid from decalcifying the tooth surface.

2. When those teeth that had been placed in solutions containing the so-called protective substances were removed and placed in acid solutions, this film must be washed away either slowly or rapidly so that the acid can reach the surface of the tooth and cause decalcification.

3. It is possible that the protection that fluorides provide is in the form of a film of a colloidal nature which appears to be more resistant. The fact that the effectiveness as a protective can be destroyed or removed by prolonged acid action, by repeated rinsing with water or by sodium hydroxide action suggests that, in some instances a fluoridal protective film may be produced rather than a true absorption of the fluoride into the internal enamel structure. Sodium hydroxide does not dissolve fluoride





## "S-10"

salts, thus if the fluoride was truly absorbed by the enamel, the sodium hydroxide should have no effect but since it had, this leads one to believe that the sodium hydroxide possibly releases the fluoride salts into solution by actually causing a destruction of the film in which they were suspended.

4. Lipase fat splitting enzymes does not seem to be capable of removing the fatty acid film on the tooth surface. Perhaps the deposition of fatty acid molecules and the removal of them by the lipase enzyme are in equilibrium. That is to say as quickly as the enzyme may remove the fatty acid film it is re-deposited on the tooth surface; however, this is merely theoretical supposition.

5. A hypothesis on oral salivary protection might be evolved from these results.

a. Certain materials present in the saliva of caries immune mouths may be capable of laying down a film on the teeth which is resistant to acid and enzyme attack.

b. These same materials may be absent in the saliva of caries susceptible mouths.

c. The characteristic acid pH. of the saliva would favour the precipitation of these materials out of the saliva onto the teeth. To prove or disprove this hypothesis a few experiments should be carried out. An outline of proposed experiments will be made at the forthcoming annual meeting.





SUPPLEMENT TO APPENDIX "S"

Project DR 18

PROPOSED EXPERIMENTAL WORK FOR

TERM 1950-51

1. Experiments to advance the study in the artificial production of dental caries in vitro, specifically related to the phase of enzymolysis.

A. Hyaluronidase To study the "Spreading Action" of hyaluronidase on enamel and dentin.

Experiment 1 Expose the surface of enamel and dentin to the action of the enzyme. Use carbon tracer.

Experiment 2 Expose the surface of enamel and dentin to the action of hyaluronidase + lactic acid. Use control.

Experiment 3 Expose the surface of enamel and dentin to the action of hyaluronidase + proteolytic enzymes. Use control.

Experiment 4 Expose the tooth to the action of hyaluronidase + lactic acid + proteolytic enzymes. Use control.

B. Tyrosinase To artificially produce a pigment similar to that found in the caries lesion.

Experiment 1 Expose the surface of enamel and dentin to the action of tyrosinase.

Experiment 2 Expose the surface of enamel and dentin to the action of tyrosinase + lactic acid.

Experiment 3 Expose the surface of enamel and dentin to the action of tyrosinase + proteolytic enzymes.

Experiment 4 Expose the tooth to the action of tyrosinase + lactic acid + proteolytic enzymes.

Experiments 2,3,4, use controls.

C. Chondrosulfatase. To study the action of a specific sulfatase on enamel and dentin.

Experiment 1 Expose the surface of enamel and dentin to the action of chondrosulfatase.





SUPPLEMENT TO APPENDIX "S"

- 2 -

Experiment 2 Expose the surface of enamel and dentin to the action of chondrosulfatase - lactic acid.

Experiment 3 Expose the surface of enamel and dentin to the action of chondrosulfatase - proteolytic enzymes.

Experiment 4 Expose the tooth to the action of chondrosulfatase - lactic acid - proteolytic enzymes.

Experiment 5 Repeat experiments 1,2,3,4, with the addition of hyaluronidase.

Experiment 6 Repeat experiments 1,2,3,4,5, with the addition of tyrosinase.

Experiments 2,3,4,5,6 use controls.

II. Experiments to study certain aspects of natural and artificial protection of teeth against caries.

Experiment 1 To study the lipolytic properties of caries-susceptible and caries-immune mixed saliva. Collect saliva specimens and add fresh olive oil (neutral fat). Measure height of oil, shake well, and leave for specified times and then measure height of oil again to determine whether any of the neutral fat has been broken down to fatty-acid. Test with Nile blue indicator for the presence of fatty-acid and neutral fat.

Experiment 2 To study the lipolytic properties of Serous Parotid saliva, from caries immune and caries susceptible mouths. Technique same as for experiment No. 1.

Experiment 3 To study the lipolytic properties of the mucous secretion from sublingual and submaxillary glands taken from caries susceptible and caries immune mouths. Technique same as for experiments No. 1 and No. 2.

Experiment 4 To determine ratio quantities of fatty-acid precipitated by acid treatment in caries immune and caries susceptible mouths from

- (a) mixed saliva
- (b) Parotid secretion
- (c) sublingual and submaxillary secretion.

Experiment 5 By means of acid treatment attempt to precipitate a protective film from caries immune saliva and caries susceptible saliva onto some extracted teeth and then subject them to acid and enzyme treatment in order to attempt to ascertain whether artificial caries can be produced in the above described environment or whether their activity is inhibited.





# SUPPLEMENT TO APPENDIX "S"

-3-

Experiment 6 Repeat experiment No. 5 but with the addition to the salivas of the following -

- (a) lipolytic enzyme
- (b) neutral fat
- (c) neutral fat and lipolytic enzyme
- (d) fatty acids

in an attempt to determine which substance or substances might have an important influence on the ability of saliva to develop or make a protective substance which could be precipitated as a film on the surfaces of teeth.

|                  |                                        |                    |          |         |         |
|------------------|----------------------------------------|--------------------|----------|---------|---------|
| DR 1             | Dr. J. J. ...                          |                    |          |         |         |
| DR 4             | Dr. H. K. Box                          |                    |          |         |         |
| DR 5             | Dr. R. L. Twible                       | 9500.00            |          |         |         |
| DR 6             | Col. E. W. Wansbrough                  | No grant received. |          |         |         |
| DR 7             | Dr. J. B. Bagnall                      | 725.00             | 394.00   | 75.00   | 70.00   |
| DR 8             | Dr. A. D. A. Mason                     | 1000.00            | 712.23   |         |         |
| DR 9             | Drs. O. J. Walker and H. R. MacLean    | 3750.00            | 529.88   | 300.00  | 120.12  |
| DR 10            | Dr. G. M. MacDonald                    | 800.00             | 244      |         |         |
| DR 11            | Dr. R. J. Godfrey                      | 6550.00            | 5542.78  | 559.07  | 559.07  |
| DR 12, 21 and 25 | Dr. A. E. R. Westman                   | 10825.00           | 10825.00 | 4200.00 | 3000.00 |
| DR 13            | Dr. M. D. Smith                        | 2505.00            | 2406.71  | 1075.30 | 100.00  |
| DR 14            | Dr. M. D. Smith                        | 2525.00            | 2402.40  |         |         |
| DR 15            | Dr. W. F. Walker                       | 6000.00            | 4250.37  | 2000.00 | 1000.00 |
| DR 16            | Dr. C. R. Bent                         | 2500.00            | 2309.78  |         |         |
| DR 17            | Dr. R. F. Shaner and Dr. H. R. MacLean | 1000.00            | 985.00   |         |         |
| DR 18            | Dr. H. K. Box                          | 2970.00            | 2375.00  | 2074.00 | 200.00  |
| DR 19            | Dr. J. K. W. Ferguson                  | 2375.00            | 2375.00  | 112.00  | 100.00  |
| DR 20            | Dr. J. Thibaud                         | 1950.00            | 1104.11  | 750.00  | 750.00  |
| DR 21            | Dr. A. E. R. Westman                   | (See DR 12)        |          |         |         |





# APPENDIX "T"

## Awards and Expenditures to Date Under Grants from the Associate Committee on Dental Research

| Project<br>No.      | Name                                    | 1945 to 31 Mar. 1949 |          | 1949 to 31 Jan. 1950 |          |
|---------------------|-----------------------------------------|----------------------|----------|----------------------|----------|
|                     |                                         | Granted              | Expended | Granted              | Advanced |
|                     |                                         | \$                   | \$       | \$                   | \$       |
| DR 1                | Dr. J.J. Rae                            | 5212.52              | 3980.38  | 1000.00              | 708.46   |
| DR 2                | Dr. G.H.W. Lucas                        | 10021.80             | 4553.93  | 325.00               | 304.26   |
| DR 3                | Dr. H.K. Box                            | 2300.00              | 275.00   | 100.00               | 75.00    |
| DR 4                | Dr. H.K. Box                            | 8600.00              | 4500.07  |                      |          |
| DR 5                | Dr. R.L. Twible                         | 9600.00              | 6975.00  |                      |          |
| DR 6                | Col. E.M. Wansbrough                    | No grant required.   |          |                      |          |
| DR 7                | Dr. J.S. Bagnall                        | 725.00               | 396.00   | 75.00                | 70.00    |
| DR 8                | Dr. A.D.A. Mason                        | 1000.00              | 912.23   |                      |          |
| DR 9                | Drs. O.J. Walker and<br>H.R. MacLean    | 3750.00              | 529.88   | 300.00               | 120.12   |
| DR 10               | Dr. G.M. MacDonald                      | 800.00               | Nil      |                      |          |
| DR 11               | Dr. R.J. Godfrey                        | 6550.00              | 4742.76  | 559.07               | 559.07   |
| DR 12, 21<br>and 25 | Dr. A.E.R. Westman                      | 10825.00             | 10090.00 | 4000.00              | 3000.00  |
| DR 13               | Dr. M.D. Smith                          | 2505.00              | 2496.53  | 1475.00              | 956.56   |
| DR 14               | Dr. M.D. Smith                          | 2625.00              | 2622.69  |                      |          |
| DR 15               | Dr. N.F. Walker                         | 6000.00              | 4956.90  | 2500.00              | 1000.00  |
| DR 16               | Dr. C.H. Best                           | 2600.00              | 2593.72  |                      |          |
| DR 17               | Dr. R.F. Shaner and<br>Dr. H.R. MacLean | 1000.00              | 400.00   |                      |          |
| DR 18               | Dr. H.K. Box                            | 2970.00              | 2370.00  | 2078.80              | 1163.80  |
| DR 19               | Dr. J.K.W. Ferguson                     | 2875.00              | 2237.08  | 314.68               | 314.68   |
| DR 20               | Dr. J. Thébaud                          | 1550.00              | 1106.13  | 750.00               | 375.00   |
| DR 21               | Dr. A.E.R. Westman                      | (See DR 12)          |          |                      |          |





| Project<br>No. (cont'd) | Name                                         | 1945 to<br>Granted | 31 Mar. 1949<br>Expended | 1949 to 31 Jan. 1950<br>Granted | Advanced |
|-------------------------|----------------------------------------------|--------------------|--------------------------|---------------------------------|----------|
|                         |                                              | \$                 | \$                       | \$                              | \$       |
| DR 22                   | Dr. C.H. Best                                | 2200.00            | 2200.00                  | 2400.00                         | 2400.00  |
| DR 23                   | Dr. C.P. Leblond                             | 2200.00            | 2200.00                  | 2200.00                         | 2200.00  |
| DR 24                   | Dr. C.H.M. Williams                          | 1420.00            | 907.00                   | 3080.00                         | 1813.00  |
| DR 25                   | Dr. A.E.R. Westman                           | (See DR 12)        |                          |                                 |          |
| DR 26                   | Dr. R.E. Moyers                              |                    |                          | 1450.00                         | 1450.00  |
| DR 27                   | Dr. J.W. Abbiss and<br>Dr. A.G. Nutlay       |                    |                          | 1000.00                         | 700.00   |
| DR 28                   | Dr. O.J. Walker and<br>Dr. H.R. MacLean      |                    |                          | 1000.00                         | 1000.00  |
| DR 29                   | Dr. M. D. Smith                              |                    |                          | 1500.00                         | 950.45   |
| DR 30                   | Dr. N. Ford Walker and<br>Dr. S.A. MacGregor |                    |                          | 1800.00                         | 1800.00  |





# APPENDIX "U"

## ASSOCIATE COMMITTEE ON DENTAL RESEARCH

### PAPERS PUBLISHED

Committee  
Paper No.

Title and Author

Reference

- 1 New Aspects of Periodontal Research  
by H. K. BOX  
Presented at National Convention of Canadian Dentists, Toronto, 29 May 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb. 1947).
- 2 Concerning the Pathogenesis of Periodontal Disease,  
by H. K. BOX  
J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg., A.C.D.R., 2 Mar. 1948).
- 3 The Effect of Various Inorganic Salts on the Solubility of Calcium Phosphate, Tooth Enamel and Whole Teeth in Lactic Acid,  
by J.J. RAE and C.T. CLEGG  
J. Dent. Res. 27: 52-53. 1948.
- 4 Changes in the Calcium and Phosphate Concentrations of Saliva and Inorganic Salt Solutions on Shaking with Calcium Phosphate,  
by J.J. RAE and C.T. CLEGG  
J. Dent. Res. 27: 54-57. 1948.
- 5 An Improved Method for Processing Acrylic Dentures,  
by D.R. CHRISTIE  
J. Can. Dent. Assoc., June, 1948. 15 pp. (App. "R", 8th Mtg., A.C.D.R., 26 Oct., 1948).
- 6 The Therapeutic Properties of Periodontal Cement Packs,  
by W.J. LINGHORNE and D.C. O'CONNELL  
J. Can. Dent. Assoc., April, 1949. 7 pp.
- 7 Phosphatase in Saliva,  
by J.T. DENTAY and J.J. RAE  
J. Dent. Res., Feb. 1949. 4 pp.





| <u>Committee<br/>Paper No.</u> | <u>Title and Author</u>                                                                                                            | <u>Reference</u>                                                 |
|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| 8                              | The Treatment of Acute Oral<br>Vincent's Infection,<br>by W.J. LINGHORNE and<br>D.F. STEDMAN                                       | J. Can. Dent. Assoc.,<br>July, 1949. 6 pp.<br>(N.R.C. No. 1699). |
| 9                              | A Comparison of Nine Local<br>Anaesthetics,<br>by H.S. HAMILTON,<br>B.A. WESTFALL and<br>J.K.W. FERGUSON                           | J. Pharmacol.<br>Expt. Therap.,<br>94: 299-307. 1948.            |
| 10                             | New Methods of Repairing<br>Acrylic Dentures Without<br>Distortion,<br>by D. R. CHRISTIE                                           | J. Can. Dent.<br>Assoc.,<br>Nov. 1949. 11 pp.                    |
| 11                             | The Corrosion of Cobalt Chromium<br>Alloys in Commercial Cleaning<br>Agents, by D.E.R. COGGLES,<br>O.J. WALKER and<br>H.R. MACLEAN | J. Can. Dent. Assoc.,<br>16: 75-82. 1950.                        |
| 12                             | The Relation between Buffering<br>Capacity, Viscosity, and Lacto-<br>bacillus Count of Saliva, by<br>J.J. RAE and<br>C. T. CLEGG   | J. Dent. Res.,<br>28: 589-593. 1949.                             |

The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

March, 1950.





APPENDIX "V"

Mr. C. A. Morrell

February 17, 1950.

February 17, 1950.

Mr. C. A. Morrell,  
Director, Food and Drug Divisions,  
Department of National Health and Welfare,  
35 John St.,  
Ottawa, Ontario.

Dear Mr. Morrell:

Following receipt of your letter of February 6th, and the enclosed copy of Dr. Katzman's report, I have discussed this matter with the Head of our Department of Dental Surgery and Anaesthesia. Therefore, the statements made in this letter represent the viewpoint of the Department teaching anaesthesia to dental students in this Faculty.

We would say that any action that may be taken relative to the percentage accuracy of anaesthetic equipment should keep in mind the long range effect it would have on the administration of general anaesthetics in dental offices. For obvious reasons dentistry cannot discharge its obligation to the public without the use of these agents.

Even with the imperfect equipment available in the early years, dentistry has used nitrous oxide for over a hundred years with a minimum of mortality. In fact the record that we have established with this drug is so good that it has never been equalled by any other group working with any other agent. On this basis we intend to continue its use until such time as the critics can demonstrate a safer or more pleasant anaesthetic for dental purposes. To date they have not done so.

We would like to draw your attention to the fact that the problem of accuracy of output is not confined to "Nitrous Oxide Anaesthetic Machines (Dental)", but applied in like measure to all such equipment, whether located in hospitals or elsewhere. We do not wish the dental equipment to be particularized in this manner.

It has taken years of work and the effort of the best brains to bring this equipment to its present state of refinement, and we have come a long way from the first





"V-2"

Mr. C.A. Morrell

DEPARTMENT OF February 17, 1950.

NATIONAL HEALTH AND WELFARE  
apparatus of Sir Frederick Hewitt to our modern machines, Much of the early equipment was crude in comparison with present day standards. Indeed, much of the apparatus still in use in England is rather primitive, but in spite of this the record is good.

Courville's work is essentially sound, but unfortunately it has been widely publicised as a reason why Nitrous Oxide should not be used by dentistry. In this connection it is only fair to note that of the twenty-six cases reported by Courville, only three or four were dental cases. The only way in which you can kill a patient with Nitrous Oxide is through asphyxia, and some of the cases as reported by Courville suggest crass ignorance on the part of the anaesthetists. Anyone who would asphyxiate a patient to the point of death with Nitrous Oxide should condemn himself, and not the agent used. It is the patient that should be read, and not the dials on the machine.

Dr. J. K. However, we would still like to see anaesthetic equipment that would provide uniform mixtures of relative accuracy, provided that manufacturing costs would not make the production of such a machine prohibitive, and we would be heartily in accord with your approaching the manufacturer on this basis. However, anaesthetic apparatus costs are already too high in relation to dental fees, and we would not wish to see the situation worsen in this respect. We must, therefore, be insistent that no action that you may initiate should end in disservice to the public through denying to them the benefits of anaesthesia for any reason whatsoever.

The next meeting of the Associate Committee on Dental Research is scheduled for Thursday, March 2nd, at which time I shall bring this matter to the attention of the Committee.

Yours sincerely,

"C.A. Morrell"  
Director, Food and Drug Divisions.

"Roy G. Ellis"  
Dean.

RGE:hc





NITROUS OXIDE AND "V-3" FIO GAS MACHINES (Dental)

DEPARTMENT OF

Introduction

NATIONAL HEALTH AND WELFARE

Nitrous oxide has been used for many years as an anesthetic by the medical and dental professions. It is safe and easy to administer. However, a group of practitioners began to question the wisdom of using an anesthetic that is administered with a low percentage of oxygen (see, "Untoward effects of nitrous oxide anesthesia," M. Courville, M.D.). In view of fact that there is the possibility of harmful damage to the human body because of lack of oxygen side anesthesia, it was decided to test the gas in use by the dental Food and Drug Divisions, 35 John Street, Ottawa, Ont. February 6, 1950.

Dr. R. G. Ellis, Dean, Ontario College of Dentistry, University of Toronto, Toronto, Ont.

Dear Doctor Ellis:

I am sending you herewith a copy of a report from Dr. J. Katzman, on a comparison of dial readings and oxygen delivered by a number of dental anaesthetic gas machines. Dr. Katzman has been on loan to this Department from the National Research Council and has now returned to the Council.

I would much appreciate your comments and those of the Associate Committee on Dental Research and the Canadian Dental Association on this report, together with suggestions for action which may be desirable.

I had thought of discussing the matter with the manufacturers and would like your views before doing so.

Sincerely yours,

"C.A. Morrell"

Director, Food and Drug Divisions.

Encl. to determine whether a machine will repeat. The uncondensable gases consisted of oxygen and the impurity of the nitrous oxide. The purity of the nitrous oxide was determined and the necessary correction was applied to the volume of the uncondensable gases to determine the oxygen volume.

Results

The results obtained from the Heidbrink machines numbered from 1 - 12 are shown in TABLE I.





## NITROUS OXIDE ANAESTHETIC GAS MACHINES (Dental)

J. Katzman

### Introduction

Nitrous oxide has been used for many years as an anaesthetic by the medical and dental professions. The gas is considered safe and easy to administer. However, with its continued use a group of practitioners began to question the wisdom of using an anaesthetic that is administered with a low percentage of oxygen (see, "Untoward effects of nitrous oxide anaesthesia" by Cyril M. Courville, M.D.). In view of fact that there is the possibility of harmful damage to the human body because of lack of oxygen during nitrous oxide anaesthesia, it was decided to test the nitrous oxide anaesthetic gas machines in use by the dental profession.

Thirteen machines were obtained for test, from Ottawa, and one machine was obtained from the Dental Faculty of Toronto University. The group was composed of twelve Heidbrink and two McKesson machines. Of the Heidbrink machines three were the new type and in service for approximately three years. The other Heidbrinks were of the old type and in service for twenty years. One McKesson was a new machine in service for two years and the one obtained from Toronto was a machine that had been in use for a long time and was reconditioned.

### Test procedure

The oxygen content of the gas mixture delivered by an anaesthetic gas machine was determined by the following method. The machine was connected to the apparatus at A, Fig. 1. The apparatus was evacuated to approximately 15 microns. The machine was set at a definite oxygen percentage as indicated by the machine oxygen dial. The manifold ST of the apparatus was flushed twice with the gas mixture and evacuated. A 100 cc. gas sample was drawn into the gas burette C and was passed slowly into the freezing vessel D, immersed in liquid nitrogen, with the Toepler pump F full of mercury. Nitrous oxide is in the solid state at liquid nitrogen temperature. The level of the mercury in arm E was marked and when it came to a definite position, the uncondensable gases in D were pumped over to gas burette G by means of the Toepler pump. A Beckman oxygen analyzer B was used to determine whether a machine will repeat. The uncondensable gases consisted of oxygen and the impurity of the nitrous oxide. The purity of the nitrous oxide was determined and the necessary correction was applied to the volume of the uncondensable gases to determine the oxygen volume.

### Results

The results obtained from the Heidbrink machines numbered from 1 - 12 are shown in TABLE I.







"V-5"

TABLE I

| Machine Setting<br>% O <sub>2</sub> | Test % O <sub>2</sub> |      |      |      |      |      |      |      |      |       |       |       |
|-------------------------------------|-----------------------|------|------|------|------|------|------|------|------|-------|-------|-------|
|                                     | No.1                  | No.2 | No.3 | No.4 | No.5 | No.6 | No.7 | No.8 | No.9 | No.10 | No.11 | No.12 |
| 7                                   | 6.0                   | 4.9  | 6.7  | 7.3  | 4.7  | 2.2  | 6.6  | 3.7  | 7.2  | 5.3   | 6.7   | 8.1   |
| 10                                  | 8.1                   | 6.7  | 10.0 | 10.8 | 9.1  | 3.5  | 9.0  | 5.4  | 9.1  | 6.8   | 9.9   | 11.2  |
| 15                                  | 12.3                  | 9.2  | 15.2 | 15.2 | 11.6 | 6.5  | 13.5 | 7.0  | 14.0 | 8.9   | 16.8  | 13.9  |
| 20                                  | 17.2                  | 12.5 | 19.7 | 19.8 | 14.7 | 10.0 | 20.1 | 8.1  | 19.0 | 10.2  | 21.8  | 20.1  |

The McKesson machine, Nargraf type, obtained in Ottawa was found to be erratic. The oxygen delivery could not be depended upon for repetition. The results obtained with this machine are shown in TABLE II.

TABLE II

| Machine Setting<br>% O <sub>2</sub> | Test % O <sub>2</sub> |     |      |      |
|-------------------------------------|-----------------------|-----|------|------|
| 5                                   | 4.5                   | 1.2 | 1.7  | 3.5  |
| 7                                   |                       |     |      |      |
| 10                                  | 6.6                   |     | 10.4 | 6.3  |
| 15                                  | 8.2                   |     | 15.7 | 7.7  |
| 20                                  | 3.2                   | 5.9 | 15.5 | 14.6 |
| 35                                  |                       |     |      | 11.3 |

The results obtained with the McKesson machine, Nargraf type, obtained from Toronto are shown in TABLE III

TABLE III

| Machine Setting<br>% O <sub>2</sub> | Test % O <sub>2</sub> |      |
|-------------------------------------|-----------------------|------|
| 5                                   | 11.1                  | 1    |
| 7                                   |                       | 19.0 |
| 10                                  | 27.8                  | 29.8 |
| 15                                  |                       | 45.5 |

### Discussion

It is obvious from the results shown in Tables I, II, and III that some machines cannot be trusted for oxygen percentage settings. An operator with machine No. 6, Table I, for example, could find himself in difficulty without appreciating the cause of the difficulty.





"V-6"

It is imperative, therefore, that some regulation be adopted that will have a measure of control over the anaesthetic gas machines. A proposed range of permissible oxygen percentage is shown in TABLE IV.

TABLE IV

| Machine<br>Setting<br>% O <sub>2</sub> | Proposed<br>Range<br>% O <sub>2</sub> |
|----------------------------------------|---------------------------------------|
| 7                                      | 6 - 8                                 |
| 10                                     | 9 - 11                                |
| 15                                     | 13.5 - 16.5                           |
| 20                                     | 18 - 22                               |

It is more important that the oxygen delivery at the low concentrations be exact than that at the higher concentrations. Therefore, a machine that is set at 7% oxygen and delivers less than 6% should be considered unsafe. Whereas, for example, a machine that is set at 15% oxygen and delivers 11% should be considered doubtful. But a machine that does not repeat within reasonable limits is definitely unsafe.





"V-7"

1. Dr. E. G. Ellis, Chairman
2. Dr. W. J. Mackenzie (ex officio)

Rep. Dental Profession

3. Dr. E. R. Box
4. Dr. E. Charron
5. Dr. M. S. Garvin
6. Dr. H. S. MacLean
7. Dr. A. E. McDougall

Rep. Royal Canadian Dental Corps

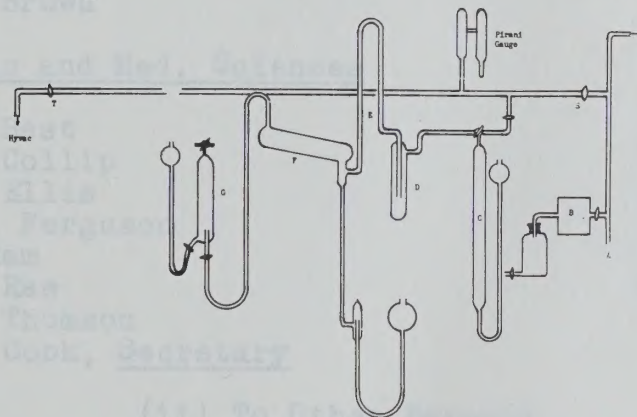
8. Col. E. M. Wainwright (ex officio)

Rep. Division of Dental Health  
Dept. National Health and Welfare

9. Dr. E. E. Brown

Rep. Basis and Med. Sciences

10. Dr. G. H. Scott
11. Dr. J. B. Collins
12. Dr. G. W. Ellis
13. Dr. J. I. W. Ferguson
14. Dr. A. W. Ross
15. Dr. J. J. Rea
16. Dr. D. L. Thompson
17. Mr. S. J. Cook, Secretary



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20. McGill - Dr. D. P. MacLean

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**Figure 1:- Apparatus used testing anaesthetic gas machines.**





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- \* 4. Dr. E. Charron 31 March, 1951 -
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- 9. Dr. H. K. Brown --

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- 11. Dr. J. B. Collip 31 March, 1952
- 12. Dr. O. W. Ellis 31 March, 1951 -
- 13. Dr. J.K.W. Ferguson 31 March, 1951 -
- 14. Dr. A.W. Ham 31 March, 1952
- 15. Dr. J. J. Rae 31 March, 1953
- \* 16. Dr. D. L. Thomson 31 March, 1953
- \* 17. Mr. S. J. Cook, Secretary --

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
OF THE  
NINTH MEETING  
OF THE  
EXECUTIVE ASSOCIATE COMMITTEE  
ON DENTAL RESEARCH

OTTAWA

16 OCTOBER, 1950







# Proceedings of the Ninth Meeting of the

## EXECUTIVE

### ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Room 238 Chateau Laurier, 8 p.m., 16 October, 1950.

#### 1. Attendance

Dr. R.G. Ellis, Chairman

Dr. E. Charron

Dr. A.W. Ham

Dr. D.L. Thomson

Mr. S.J. Cook, Secretary

#### 2. Inquiry Regarding an Application

THE CHAIRMAN reported on an inquiry which had been received, in which it was asked whether the Committee would consider an application for a grant from a private research organization. After some discussion it was AGREED that further information should be obtained, and that if the Chairman thought it advisable to proceed further with the matter, he should discuss the general policy with President C.J. Mackenzie.

#### 3. Notification to Grantees

THE CHAIRMAN asked the Secretary to report on the financial position of the Committee. The Secretary said that quite a few grantees had not claimed the full amount of their awards and it was not known whether the grantees would want the full amounts or not. It was AGREED that the Secretary should write a letter to each grantee asking how much more money they expected to be able to use before 31 March and pointing out that if there is any surplus in their grant that will be unexpended during this period it should be returned to the Committee by 31 December, 1950, in order that such moneys might be made available for other grants.

#### 4. Grant to Dr. Rae under DR 1

In a letter from Dr. Rae he pointed out that the Committee had agreed to advance him \$500 under DR 1 for the employment of a full-time assistant during the summer months June-September at \$125 per month but that he had been unable to procure a suitable assistant at that salary. However, Mr. C.T. Clegg, his part-time assistant, had been able to devote full time to the research project for the months June, July and August, and Dr. Rae requested that he be allowed to pay Mr. Clegg \$300 (\$100 a month) of the \$500 allotted to him for salaries to be paid to Mr. Clegg for his summer work. It was agreed to APPROVE his recommendation.

The Meeting adjourned at 10:30 P.M.







5. Grant to Dr. Norma Ford Walker under DR 30

DR. NORMA FORD WALKER had written asking that a further grant be made to enable her to employ Dr. W.L. Liu from October 1950 to March 1951 (6 months at \$200 per month), total \$1200. This was a renewal of her original request for a grant which had been reduced by the Executive and the Committee at the March meeting from \$2600 for the year to \$1200 for salaries plus \$200 for supplies, a total of \$1400 for the period April-September 1950.

On consideration of the present financial position of the Committee it was AGREED to recommend this further award of \$1200 to Dr. Walker for the period ended 31 March 1951.

6. Fellowship Application from Lesley A. Gill

THE CHAIRMAN presented an application for a fellowship which he had received on 13 October 1950, through the Chairman of the Research Committee of the Canadian Dental Association. The application had been made out under date of 13 May 1950. As the application was incomplete the Secretary was instructed to write for further information and to point out that the deadline for applications is 1 February in each year. The Secretary was also to get a transcript of the candidate's record and to obtain further information as to whether he proposed to give only part time or full time to the project.

7. Estimates, 1951-52

THE CHAIRMAN said that the National Research Council would want advice very shortly on the financial requirements of the Associate Committee on Dental Research for the next fiscal year. He said that for the present year and several years past the Committee had received \$35,500 which included \$27,500 for grants, \$5,000 for fellowships, \$2500 for travel, and \$500 for contingencies. It was AGREED to recommend that the appropriation for 1951-52 be \$40,500; this includes an increase of \$5,000 in the item for grants-in-aid at a total of \$32,500.

8. New Members of the Committee

THE CHAIRMAN pointed out that Dr. C.B. Stewart, representative in the basic sciences, had resigned and that Dr. H.K. Box, a representative of the dental sciences, had also been compelled to retire from membership in the Committee because of ill health. On consideration of these two vacancies it was AGREED to recommend that Dr. J.B. Macdonald, Assistant Professor of Bacteriology, Faculty of Dentistry, University of Toronto be appointed for the remaining period of Dr. C.B. Stewart's term of office which expires 31 March, 1951.

It was AGREED to recommend the appointment of Dr. J.S. Bagnall, Dean, Faculty of Dentistry, Dalhousie University, Halifax, N.S., for the remaining period of the appointment of Dr. H.K. Box which expires 31 March, 1952.

The Meeting adjourned at 10:30 P.M.







Members of the Associate Committee on Dental Research  
and its Executive, with their terms of appointment

Representing the Division of Dental Health  
Department of National Health and Welfare

|      |                                                                                                           | <u>Term to Expire</u> |
|------|-----------------------------------------------------------------------------------------------------------|-----------------------|
| ★ 1. | Dr. R.G. Ellis, Chairman,<br>Dean of the Faculty of Dentistry,<br>University of Toronto,<br>Toronto, Ont. | 31 March, 1951        |

|    |                                                                                             |   |
|----|---------------------------------------------------------------------------------------------|---|
| 2. | Dr. C.J. Mackenzie(ex officio),<br>President,<br>National Research Council,<br>Ottawa, Ont. | - |
|----|---------------------------------------------------------------------------------------------|---|

Representing the Dental Profession

|    |                                                                                      |                |
|----|--------------------------------------------------------------------------------------|----------------|
| 3. | Dr. H.K. Box,<br>Prof. of Periodontology,<br>University of Toronto,<br>Toronto, Ont. | 31 March, 1952 |
|----|--------------------------------------------------------------------------------------|----------------|

|      |                                                                                                        |                |
|------|--------------------------------------------------------------------------------------------------------|----------------|
| ★ 4. | Dr. E. Charron,<br>Dean of the Faculty of Dental Surgery,<br>University of Montreal,<br>Montreal, Que. | 31 March, 1951 |
|------|--------------------------------------------------------------------------------------------------------|----------------|

|    |                                                                                                                         |                |
|----|-------------------------------------------------------------------------------------------------------------------------|----------------|
| 5. | Dr. M.H. Garvin,<br>Editor-in-Chief,<br>Journal of the Canadian Dental Assoc.,<br>314 Somerset Bldg.,<br>Winnipeg, Man. | 31 March, 1953 |
|----|-------------------------------------------------------------------------------------------------------------------------|----------------|

|    |                                                                                                                             |                |
|----|-----------------------------------------------------------------------------------------------------------------------------|----------------|
| 6. | Dr. H.R. MacLean,<br>Lecturer in Operative Dentistry,<br>Faculty of Dentistry,<br>University of Alberta,<br>Edmonton, Alta. | 31 March, 1953 |
|----|-----------------------------------------------------------------------------------------------------------------------------|----------------|

|    |                                                               |                |
|----|---------------------------------------------------------------|----------------|
| 7. | Dr. R.H. McDougall,<br>1505 Hampshire Road,<br>Victoria, B.C. | 31 March, 1952 |
|----|---------------------------------------------------------------|----------------|

Representing the Royal Canadian Dental Corps

|      |                                                                                                                                                         |   |
|------|---------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| ★ 8. | Col. E.M. Wansbrough(ex officio),<br>Director General of Dental Services,<br>Royal Canadian Dental Corps,<br>Dept. of National Defence,<br>Ottawa, Ont. | - |
|------|---------------------------------------------------------------------------------------------------------------------------------------------------------|---|





Representing the Division of Dental Health  
Department of National Health and Welfare

9. Dr. H.K. Brown, (ex officio),  
Chief, Dental Health Division,  
Dept. of National Health and Welfare,  
Ottawa, Ont.

Representing the Basic and Medical Sciences

10. Dr. C.H. Best,  
Director, Banting and Best Dept. of  
Medical Research,  
University of Toronto,  
Toronto, Ont. 31 March, 1953
11. Dr. J.B. Collip,  
Dean of the Faculty of Medicine,  
University of Western Ontario,  
London, Ont. 31 March, 1952
12. Dr. O.W. Ellis,  
Dept. of Metallurgy,  
Ontario Research Foundation,  
Toronto, Ont. 31 March, 1951
13. Dr. J.K.W. Ferguson,  
Head of the Dept. of Pharmacy  
and Pharmacology,  
University of Toronto,  
Toronto, Ont. 31 March, 1951
14. Dr. A.W. Ham,  
Prof. of Anatomy,  
Faculty of Medicine,  
University of Toronto,  
Toronto, Ont. 31 March, 1952
15. Dr. J.J. Rae,  
Dept. of Chemistry,  
University of Toronto,  
Toronto, Ont. 31 March, 1953





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16. Dr. C.B. Stewart, 31 March, 1951  
Faculty of Medicine,  
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Halifax, N.S.

\* 17. Dr. D.L. Thomson, 31 March, 1953  
Chairman of the Dept. of  
Biochemistry,  
McGill University,  
Montreal, Que.

\* 18. Mr. S.J. Cook, Secretary,  
Public Relations Branch,  
National Research Council,  
Ottawa, Ont.

10. Dr. A.W. Rae  
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13. Dr. E.H. McDougall  
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| 2. Dr. C.H. Best                   | 11. Dr. C.J. Mackenzie<br>(ex officio)   |
| * 3. Dr. H.K. Box                  | 12. Dr. H.R. MacLean                     |
| 4. Dr. H.K. Brown (ex officio)     | 13. Dr. R.H. McDougall                   |
| 5. Dr. E. Charron                  | 14. Dr. J.J. Rae                         |
| 6. Dr. J.B. Collip                 | * 15. Dr. C.B. Stewart                   |
| 7. Dr. O.W. Ellis                  | 16. Dr. D.L. Thomson                     |
| 8. Dr. J.K.W. Ferguson             | 17. Col. E.M. Wansbrough<br>(ex officio) |
| 9. Dr. M.H. Garvin                 | 18. Mr. S.J. Cook, <u>Secretary</u>      |

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
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TWELFTH MEETING  
OF THE  
ASSOCIATE COMMITTEE  
ON DENTAL RESEARCH

OTTAWA

16 - 17 OCTOBER, 1950







## TABLE OF CONTENTS

- 2 -

### Page

|                                              |    |
|----------------------------------------------|----|
| 1. Attendance .....                          | 1  |
| 2. Chairman's Remarks .....                  | 1  |
| 3. Minutes of the 11th Meeting .....         | 1  |
| 4. Business Arising out of the Minutes ..... | 2  |
| 5. Ownership of Publication . . . . .        | 3  |
| 6. DR 1 - Dr. J. J. Rae .....                | 3  |
| 7. DR 2 - Dr. G.H.W. Lucas .....             | 4  |
| 8. DR 12 - Dr. A.E.R. Westman .....          | 4  |
| 9. DR 13 - Dr. M. Doreen Smith .....         | 4  |
| 10. DR 15 - Dr. Norma Ford Walker .....      | 4  |
| 11. DR 21 - Dr. A.E.R. Westman .....         | 4  |
| 12. DR 22 - Dr. C. H. Best .....             | 4  |
| 13. DR 23 - Dr. C. P. Leblond .....          | 5  |
| 14. DR 24 - Dr. C.H.M. Williams .....        | 5  |
| 15. DR 25 - Dr. A.E.R. Westman .....         | 5  |
| 16. DR 26 - Dr. R. E. Moyers .....           | 6  |
| 17. DR 27 - Dr. J.W. Abbiss .....            | 6  |
| 18. DR 28 - Dr. O. J. Walker .....           | 6  |
| 19. DR 29 - Dr. M. Doreen Smith .....        | 7  |
| 20. DR 30 - Dr. Norma Ford Walker .....      | 7  |
| 21. DR 31 - Dr. L. B. Jacques .....          | 8  |
| 22. DR 32 - Dr. A. W. Ham .....              | 9  |
| 23. DR 33 - Dr. H. A. Hunter .....           | 10 |
| 24. DR 34 - Dr. A. D'Iorio .....             | 10 |
| 25. DR 35 - Dr. J. B. Macdonald .....        | 10 |





# TABLE OF CONTENTS

- 2 -

|     |                                                                           |    |
|-----|---------------------------------------------------------------------------|----|
| 26. | Attendance (Second Session).....                                          | 11 |
| 27. | Telegram from Dr. Bagnall .....                                           | 11 |
| 28. | Report by the Chairman on his Visit to Australia<br>and New Zealand ..... | 12 |
| 29. | Finances.....                                                             | 12 |
| 30. | DR 29 Dr. M.D. Smith (Additional Grant) .....                             | 15 |
| 31. | DR 1 Dr. J. J. Rae .....                                                  | 15 |
| 32. | DR 30 Dr. Norma Ford Walker .....                                         | 15 |
| 33. | Finances (1951-52) .....                                                  | 16 |
| 34. | Membership of the Committee .....                                         | 16 |
| 35. | Air Travel .....                                                          | 17 |
| 36. | Fellowship Applications .....                                             | 17 |
| 37. | New Research Projects .....                                               | 17 |
| 38. | News Report of Meeting .....                                              | 18 |
| 39. | List of Members with addresses.....                                       | 18 |
| 40. | Date of Next Meeting .....                                                | 18 |

## Appendices

|              |                                                                                                                                                                      |
|--------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Appendix "A" | Entry of phosphate and carbonate salts into teeth<br>as shown with the help of radio-phosphorus and<br>radio-carbon, DR 23                                           |
| Appendix "B" | Investigation of methods for use of methyl metha-<br>crylate in direct dental fillings, DR 25                                                                        |
| Appendix "C" | An investigation of the mechanism of repair of<br>the supporting structures of the teeth, DR 22                                                                      |
| Appendix "D" | Investigation of the effect of adrenal cortical<br>hormone imbalances on the various connective tissue<br>structures of the growing incisor teeth of animals, DR 32. |
| Appendix "E" | Experimental fusospirochetal infection with<br>mixtures of pure cultures, DR 35                                                                                      |

Grantees or Assistants invited to report personally  
(1st to 12th Meeting as revised).

Initial Distribution.





# TABLE OF CONTENTS

- 3 -

## Appendices (cont'd)

- Appendix "F"      Studies of calcium metabolism in tooth with the aid of  $\text{Ca}^{45}$ , DR 34
  - Appendix "G"      A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia, DR 2
  - Appendix "H"      Investigation of materials for use as cements for methyl methacrylate inlays, DR 12
  - Appendix "I"      Investigation of repair techniques for methacrylate dentures, DR 21
  - Appendix "J"      Chemical mechanisms in dental caries, DR 1
  - Appendix "K"      A study of the calcifying potential of calcium oleate on the dental pulp, DR 33
  - Appendix "L"      A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth, DR 27
  - Appendix "M"      An investigation of nerve block, DR 31
  - Appendix "N"      Biological absorption of fluorine, DR 29
  - Appendix "O"      The study of the closure of space following premature loss of deciduous teeth, DR 30
  - Appendix "P"      Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to pabulum history of the children's diet, DR 13
  - Appendix "Q"      A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches, DR 15
  - Appendix "R"      Measurement of physiologic forces in the mouth, DR 26
  - Appendix "S"      Development of a method for determining fluorine by means of photo-electric colorimeter, DR 28
  - Appendix "T"      Associate Committee on Dental Research, Papers published.
  - Appendix "U"      The effects of hard and soft diets on the supporting structures of the teeth, DR 24
  - Appendix "V"      Report by the Chairman on his visit to Australia and New Zealand.
  - Appendix "W"      Grantees or Assistants invited to report personally (in the 12th Meeting as revised).
- Initial Distribution.





# NATIONAL RESEARCH COUNCIL

## Proceedings

### Twelfth Meeting

of the

#### ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,  
9:30 a.m., 16-17 October, 1950

#### 1. Attendance

Dr. R. G. Ellis, Chairman

Dr. C. H. Best

Dr. H. K. Brown

Dr. E. Charron

Dr. M. H. Garvin

Dr. A. W. Ham

Dr. H. R. MacLean

Dr. R. H. McDougall

Dr. J. J. Rae

Dr. D. L. Thomson

Mr. S. J. Cook, Secretary

#### By Invitation

Dr. W. J. Linghorne

Dr. Willa L. Liu

Dr. J. B. Macdonald

Mr. G. J. Millar

Dr. H. H. Saunderson

Dr. Norma Ford Walker

Apologies for their absence were received from -  
Dr. J. B. Collip, Dr. O. W. Ellis, and Col. E.M. Wansbrough.

#### 2. Chairman's Remarks

THE CHAIRMAN said that the Committee had been deprived at this Session of the presence of some of its members owing to the fact that the annual meeting of the Canadian Physiological Society was being held in Ottawa at this time. He welcomed representatives of the grantees who had come to present their reports and in this connection mentioned Dr. Linghorne, Dr. Liu, Dr. Macdonald, Mr. Millar, and Dr. Walker.

#### 3. Minutes of the 11th Meeting

It was MOVED by Dr. Charron and SECONDED by Dr. Thomson,

That the Minutes of the 11th Meeting having been  
circulated be taken as read and approved.

CARRIED



#### 4. Business Arising out of the Minutes

##### (i) Report on Distribution of Trends in Caries Research (Min. 4, 11th Mtg.)

THE SECRETARY reported that 4876 copies of Trends in Caries Research had been distributed. Of this number 4600 were sent to members of the Canadian Dental Association, 16 copies to Committee members, 10 to provincial departments of health in Canada, 36 to professors of physiology and biochemistry in Canadian universities, 80 to libraries of dental schools as follows:-

Canada 5, United States 49, British Isles 19, Australia 5, New Zealand 1, South Africa 1.

Additional distribution on request included 52 copies to the Secretary, Canadian Dental Association; 30 copies taken by the Chairman to Australia; and the following requests: Canada 5, United States 15; British Isles 14; Australia 6; South Africa 12.

THE SECRETARY has 140 copies in stock.

Total cost of publishing this booklet was \$383.40 or seven and two-third cents per copy.

##### (ii) Bibliography on Caries Research (Min. 4, 11th Mtg.)

The complete manuscript of the Bibliography was retyped and published in a seven-by-ten inch volume of 557 pages. The review material was collected under fifteen main divisions and 130 subdivisions. Some 2300 books and articles are listed in the Bibliography. The volume has been priced at \$10 per copy. While only 100 copies had been ordered in the first instance the edition had been increased on the authority of the Chairman to 200 copies when it appeared that the printing of this number was warranted. The total cost of the 200 copies was \$4622.40 of which \$1388.88 had been paid out of the Committee's 1949-50 funds leaving a balance of \$3233.52 to be paid out of 1950-51 appropriation. It was noted that the Committee at its 11th Meeting had agreed to send several copies of the complete Bibliography to each of the Canadian dental schools as well as single copies to selected dental schools in the United States and Great Britain.

After some discussion it was AGREED to limit the initial complimentary distribution to a single copy to each Institution.

It was MOVED by Dr. Rae and SECONDED by Dr. Garvin,

That a complimentary copy of the Bibliography on Caries Research be sent to the Library of each Canadian dental school and to a limited number of organizations comparable to the Associate Committee on Dental Research in other countries and that review copies be sent to the leading dental journals.

CARRIED.



In discussion it was suggested that in the letters to editors, information should be given regarding the source and price of the volume.

It was also AGREED that steps should be taken to publicize the publication in appropriate places so that information regarding the book might reach as many students of caries research as possible. To this end it was suggested that circulars be sent to medical and dental schools and to twenty or thirty journals.

Distribution of the book was left to the Chairman and Secretary to work out. A sample copy was shown to the members.

#### 5. Ownership of Publication

It was MOVED by Dr. Ham and SECONDED by Dr. Thomson,

That the necessary steps be taken to protect the Committee in regard to the distribution of the book Bibliography on Caries Research and that legal advice be secured as to the Committee's rights in the matter and that a release be obtained from Dr. Bagnall of his interest in the publication.

CARRIED.

#### Consideration of Reports from Grantees

#### 6. DR 1 - Dr. J. J. Rae

##### "Chemical mechanisms in dental caries"

DR. RAE presented a summary of work done during the last six months, an outline of proposed work and an interim report (No.10) in two sections - (a) Ammonia production in saliva and (b) An inhibitory substance for the growth of l.b.a. in tooth enamel, all of which appear in

APPENDIX "J"

DR. THOMSON questioned an item which appears in table IV on page "J-4" and which shows 24.5 mg%  $\text{NH}_3\text{-N}$  and 17.5 mg% N.P.N.

DR. RAE promised to look into this item and advise Dr. Thomson and the Secretary whether any change should be made in the figures.

DR. GARVIN said that the work on ammoniated dentifrices was of considerable interest to Canadians and he expressed the view that this work, done in a Canadian laboratory, should be published in a Canadian journal.

DR. RAE said that his earlier work had been published in the Journal of Dental Research and since his present study was of rather greater interest to research workers than to those in clinical practice he had thought the Journal of Dental Research would be a more suitable medium than the Journal of the Canadian Dental Association but he agreed that publication of Canadian work should be made in Canadian journals as a matter of general policy.



DR. HAM expressed the view that research papers should, in general, be published in research journals but agreed that practical results should appear in clinical journals.

7. DR 2- Dr. G.H.W. Lucas

"A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia"

Work had been resumed on this project in May. From June to September Dr. Smithurst was in England collecting information on nitrous oxide anaesthesia but he expected to resume his studies in Canada in October. A brief report appears in APPENDIX "G"

8. DR 12 - Dr. A.E.R. Westman

"Investigation of materials for use as cements for methyl methacrylate inlays"

Work on this project has been held in abeyance pending developments in the closely-related project DR 25 on direct acrylic fillings. APPENDIX "H"

9. DR 13 - Dr. M. Doreen Smith

"Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to Pabulum history of the children's diet"

A summary and a report appear in APPENDIX "P"

10. DR 15 - Dr. Norma Ford Walker

"A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches"

DR. WALKER presented a report which appears in APPENDIX "Q"

She illustrated her talk with a number of slides.

11. DR 21 - Dr. A.E.R. Westman

"Investigation of repair techniques for methacrylate dentures"

A summary and a final report No. 5002 were read. These appear in APPENDIX "I"

12. DR 22 - Dr. C.H. Best

"Part 1 - An investigation of the mechanism of repair of the supporting structures of the teeth;  
Part 2 - Fusospirochetal infection and pre-existing inflammation"

DR. LINGHORNE presented this report which appears in

APPENDIX "C"



DR. GARVIN said he thought this work was the most important field of research in caries as periodontal disease is the principal cause of loss of teeth. He repeated his suggestion that work of this importance done in Canadian laboratories should be published in a Canadian Journal. He thought it was important that original papers based on work of this kind should be presented in a Canadian publication because only in this way could Canadian prestige be established in the research field. Outside organizations which find Canadian research papers published in foreign journals are inclined to think that it is not necessary for them to subscribe to Canadian literature in order to keep up to date but this situation could be remedied if more Canadian research workers published their results in Canadian journals.

DR. LINGHORNE said that his work, like that of Dr. Rae, appeared to be of more interest to research workers than to clinical workers and he had therefore proposed to publish his results in a research journal; however, he would take note of Dr. Garvin's suggestions and see whether a suitable paper could be prepared for publication in the Journal of the Canadian Dental Association.

DR. HAM suggested that it would add to the value of the research results if celloidin sections could be made, especially in the horizontal plane. He thought it might be worthwhile to train another technician in this field if there seemed to be more work than one could do.

13. DR 23 - Dr. C. P. Leblond

"Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon"  
Report by Dr. Leblond appears in APPENDIX "A"

14. DR 24 - Dr. C.H.M. Williams

"The effect of hard and soft diets on the supporting structures of the teeth"  
A summary report appears in APPENDIX "U"

15. DR 25 - Dr. A.E.R. Westman

"Investigation of methods for use of methyl methacrylate in direct dental fillings"  
A summary of the work and report No. 5001 and report No. 5002 appear in APPENDIX "B"

DR. MACLEAN questioned the accuracy of a statement in para. 3 page "B-2" of report 5002 relating to figure 1. He thought that the graph was correct but that the wording of the paragraph was not in conformity with the evidence given in the graph.



DR. HAM wondered who was doing the pathology on the photomicrographs shown on pages B-7 et seq and thought it possible that the pictures on B-7 might be wrongly labelled. He pointed out for example, that in figure 4, both sides seemed about the same while in figure 5 under the high powered magnification the label described the material as "secondary dentin". He was not familiar with the development of secondary dentin in dogs and he felt that unless the pathology was being done by someone who was competent in this field, errors might creep in.

16. DR 26 - Dr. R. E. Moyers

"Measurement of physiological forces in the mouth"

A brief review of this work appears in APPENDIX "R"

It is hoped that a complete report including a statistical analysis of the clinical implications will be ready for presentation at the Spring meeting of the Committee.

THE CHAIRMAN suggested that Dr. Moyers might be invited to attend the next meeting and to present his report in person.

17. DR 27 - Dr. J. W. Abbiss and Dr. A.G. Nutlay

"A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth"

A summary report appears in APPENDIX "L"

THE SECRETARY said that the grantees had submitted a coloured plate of the two figures but that it had only been possible to reproduce the outline sketches. He passed the colour plates around for inspection.

Attention was directed to para. 1 entitled "Enamel". In line 4 it appeared that some words had been omitted after "celloidin" and before "decalcification". The Secretary was directed to bring these items to the grantee's attention.

18. DR 28 - Dr. O. J. Walker

"Development of a method for determining fluorine by means of a photo-electric colorimeter"

DR. MACLEAN presented a report on this subject which appears in

APPENDIX "S"

He said that Dr. Walker now has a part-time assistant. He said also that he thought a copy of Mr. Martin's thesis referred to in the report should be sent to the Committee as part of its records. He promised to arrange to have this done. The opinion was expressed that the thesis, when received, might be loaned to Dr. M. Doreen Smith since she is working in a related field and has had some difficulty in making fluorine determinations.



19. DR 29 - Dr. M. Doreen Smith  
"Biological absorption of fluorine"

A summary and a complete report appear in APPENDIX "N"  
THE CHAIRMAN recalled that as noted in minute 39 of the 11th Meeting it had been decided not to make a further grant to Dr. M. Doreen Smith under DR 29. However, on being advised of this decision Dr. Smith pointed out that certain parts of the work under this grant had to be done when subjects and rooms for their accommodation were available. This time happened to be January, February, March and part of April 1950. At the end of this period 5 sets of balanced studies and certain fluorine analysis would have to be made. The samples had been stored in the Connaught Laboratories and she pointed out that if a salary for Miss Ham could be provided until the end of June together with a small amount of chemicals it would be possible to complete the investigation and present a report in October 1950. She asked therefore for a further grant of \$375 for this purpose.

By letter ballot the Executive on the recommendation of the Chairman approved this further grant, and as a consequence the report in Appendix "N" was prepared. However, in view of the indefinite results in the report and the statement that "The results of the completed study will be reported later" it was suggested that the Secretary should write to inquire where this investigation now stands. Is it complete or did the difficulty encountered in the analyses preclude the reaching of a satisfactory conclusion?

(The Secretary reported that the paper referred to in Dr. Smith's report entitled "Fluoride Studies related to the Human Diet" had been received and would be included in the Committee's list of publications.)

20. DR 30 - Dr. Norma Ford Walker

"The study of the closure of space following premature loss of deciduous teeth"

DR. W.L. LIU presented the report on this subject which appears in APPENDIX "O". She also showed several lantern slides.

DR. MACDOUGALL inquired whether any other factors are involved that limit the changes or whether only growth and development are concerned.

THE CHAIRMAN enlarged the question to include all other variable factors in this problem and asked what information was coming out of this work that would be of value to the general practitioner.



DR. LIU said that unfortunately only 35 cases are under examination whereas Hellman had been able to work on more than 200 cases. She thought that the results obtained indicated that examination every three months would be most useful. Space maintainers if they were to be used should be put in shortly after the eruption of the teeth. The purpose of the study is to evaluate earlier work reported in the literature. She hoped that the investigation could be completed next year.

DR. MACDONALD inquired regarding the method of measuring arch width and asked whether Dr. Liu had any theory for mesial movement of the upper molar and mesial tipping of the lower molar.

DR. LIU showed some of her slides again and made further reference to data in her report.

DR. WALKER said that the difference between Hellman's results and Dr. Liu's data might be due to racial differences in the subjects examined. A large Jewish source was available to Dr. Hellman whereas the cases under examination by Dr. Liu were of Scotch, Irish and English origin.

THE CHAIRMAN inquired whether it might not be possible to prepare a paper on this work which would be suitable for publication in the Journal of the Canadian Dental Association. Such a paper should not present too much research data but should place emphasis on the clinical features.

DR. WALKER said that she thought it should be possible to prepare two such articles and she would see that this was done.

THE CHAIRMAN suggested also that a copy of the thesis being prepared by Dr. Liu on the results of this investigation should be filed with the Committee.

21. DR 31 - Dr. L. B. Jacques and Mr. G.J. Millar

"An investigation of nerve block"

A summary and an interim report appear in APPENDIX "M"

MR. G.J.MILLAR, who was present, reviewed the work that had been done to date. He said the investigation had arisen from a suggestion made by Dr. M.J. Macdonald of Saskatchewan whose letter to the Editor of the Journal of the Canadian Dental Association had been circulated for comment.

DR. MACDONALD had turned the problem over to Dr. Jacques and Mr. Millar. It appeared that one answer is that the technique is not adequate. In case the first injection does not reach the nerve, a second or third injection may be needed to secure nerve block. Further work on technique therefore seems to be indicated. Failure of nerve block is also most likely to occur in hyperaemic pulp and he thought that the sympathetic fibres should be blocked first. He could not say why failure to obtain nerve block does occur. It has been shown



that nerve impulses will jump a block under certain conditions. The technique aims at the point where the inferior nerve enters the mandible. He suggested that injection higher up might be tried with a view of blocking a longer nerve. He was keeping in close contact with local dentists and doctors in the pursuit of this study.

DR. CHARRON asked whether we know how an anaesthetic works. He said that very few failures do occur and that these happen only when the technique or selection are bad.

DR. GARVIN said that Mr. Millar had referred to the publication of reports including those by Austin of the Mayo Clinic. He had happened to be at the Mayo Clinic at one time when an operator failed to obtain anaesthesia on initial injection using a two per cent solution. The operator had changed to the use of a four per cent solution as he found that the stronger solution was necessary in order to get penetration. He thought therefore it was necessary to keep records not only of the quantities used but of the strength of the solutions as well. He noted that monacaine is coming on the market in stronger solutions now.

DR. CHARRON thought the percentage strength of the solution should not be changed but that increased quantities of the same strength might be used.

MR. MILLAR said that where two or three teeth have to be extracted a first injection may be satisfactory for the first tooth but not be adequate for the second and third tooth. Hyperaemic pulp causes failure.

DR. THOMSON said that as long as second or third injections work the reason for the initial failure will not be known.

MR. MILLAR said the anatomy department is being interested in this problem.

22. DR 32 - Dr. A.W. Ham

"Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals"

DR. HAM presented the short summary stating that it had been decided it would be more convenient to undertake this research in the Fall. He has started one batch of animals from which the adrenal glands had been removed and certain experiments are now underway.

Summary appears in

APPENDIX "D"



23. DR 33 - Dr. H. A. Hunter

"Study of the calcifying potential of calcium oleate on the dental pulp"

In this case again work has just been undertaken and a few preliminary experiments made. APPENDIX "K"

DR. LINGHORNE said that during the early summer the number of animals kept for experimental purposes is reduced to a minimum and additional animals are secured in the Fall.

DR. RAE referred to the last three words of the summary "known calcifying agents" and said that he did not know that calcium oleate was regarded as a calcifying agent.

DR. HAM said that the reference was to various calcium salts that had been used in all early work on calcification. Considerable work had been done on the use of phosphates in vitro and references to this work should be useful in the present study.

DR. MACDONALD said he thought Dr. Hunter intended to use the hexaphosphate as well as calcium salts.

THE CHAIRMAN said he would try to have Dr. Hunter present at the next meeting of the Committee to report on this subject and to show samples of his sections.

24. DR 34 - Dr. A. D'Iorio

"Studies of calcium metabolism in tooth with the aid of  $Ca^{45}$ "

DR. CHARRON presented a summary and an interim report on this work which appear in APPENDIX "F"

He suggested and it was agreed that Dr. D'Iorio might be invited to attend the next meeting of the Committee to present his report.

25. DR 35 - Dr. J. B. Macdonald

"Experimental fusospirochetal infection with mixtures of pure cultures"

DR. MACDONALD was present in person and reported on his work. His summary and the complete report appear in APPENDIX "E"

DR. GARVIN questioned the statement that the organisms do not change in virulence but was assured that this was the case.

DR. RAE asked how the researchers expected to obtain dialytic action when the same media were used on both sides of the membrane.



DR. MACDONALD said that he thought bacteria might develop which would produce a change in potential that might give rise to dialysis.

DR. RAE said that it appeared from Dr. Macdonald's work that caries is caused by three or four organisms. If the present theory of acidity is accepted all factors must be considered. He himself was not satisfied that lacto bacillus causes caries.

DR. HAM said that if virulence does not change, communicability might depend on the amount of infection that is suddenly introduced into a mouth.

DR. MACDONALD said that this was an open question as yet.

The Meeting adjourned at 5:15 P.M.

A Meeting of the Executive was held at 8 P.M.

The Second Session of the Committee Meeting began at 9 A.M. Tuesday, 17 October.

## SECOND SESSION

9 A.M., Tuesday 17 October, 1950.

### 26. Attendance

Dr. R. G. Ellis, Chairman  
Dr. H. K. Brown  
Dr. E. Charron  
Dr. M. H. Garvin  
Dr. A. W. Ham  
Dr. H. R. MacLean  
Dr. R. H. McDougall  
Dr. J. J. Rae  
Dr. D. L. Thomson

Mr. S. J. Cook, Secretary

### 27. Telegram from Dr. Bagnall

THE CHAIRMAN said that a complimentary copy of the Bibliography on Caries Research had been sent to Dr. Bagnall and the following telegram had just been received "Very much pleased with book, best wishes for good meeting".

Subsequently the Secretary received a letter from Dr. Bagnall which contained the following paragraph: "I think that the printer has done an excellent job and it certainly exceeded my expectations. If it helps to further the work of dental research, I will be very happy. I have gotten a great deal out of it myself and am now working on a set of notes for our students in the hope that I can use the lessons from this to give them a better appreciation of the methods and aims of research".



It was MOVED by Dr. Garvin and SECONDED by Dr. MacLean,

That the Associate Committee on Dental Research, having examined the published volume containing the abstracts of papers on caries research and the long list of quoted books and articles on which the review is based, desires to place on record its high appreciation of the services rendered by the author, Dr. J. S. Bagnall, both to the Committee and to all research workers interested in the study of dental caries, to whom this book will be a most valuable reference.

The Committee expresses the hope that the usefulness of this monumental compilation and review to dental research workers in Canada and abroad will afford the author a measure of compensation for his unselfish and devoted service in the preparation of this publication. CARRIED.

28. Report by the Chairman on his Visit to Australia and New Zealand

THE CHAIRMAN said that he had been privileged during the summer to spend some time in Australia and New Zealand and while there he had gained some knowledge regarding the development of dental research in these two Dominions. He had prepared a brief report which he thought would be of interest to the members of this Committee and which he then read. This report appears in full in APPENDIX "V"

DR. THOMSON speaking for the Committee expressed their gratitude for this very interesting report.

29. Finances, 1950-51 (Min. 36, 11th Mtg.)

THE SECRETARY reported on the financial standing of the Committee as at 30 September 1950 as follows:

|                          |             |
|--------------------------|-------------|
| Funds available, 1950-51 | \$35,500.00 |
| Expenditures             | 14,024.70   |
| Commitments              | 17,285.97*  |
| Free balance             | 4,299.33    |

(\* Includes unpaid balance of \$3233.52 on printing of Bibliography on Caries Research)



THE SECRETARY also reported the financial standing of the grants made by the Committee as of 30 September as follows:-

| <u>Grantee</u>                                | <u>Amount Awarded</u> | <u>Balance Payable</u> |
|-----------------------------------------------|-----------------------|------------------------|
| DR 1 Dr. J.J. Rae                             | 950.00                | 650.60                 |
| DR 2 Dr. G.H.W Lucas                          | 1550.00               | 895.74                 |
| DR 12 Dr. A.E.R. Westman<br>25                | 3000.00               | 2250.00                |
| DR 13 Dr. M.D. Smith                          | 1475.00               | 1174.85                |
| DR 15 Dr. N.F. Walker                         | 800.00                | -                      |
| DR 22 Dr. C.H. Best                           | 3800.00               | -                      |
| DR 23 Dr. C.P. Leblond                        | 2400.00               | 400.00                 |
| DR 24 Dr. C.H.M. Williams                     | 1735.00               | 1735.00                |
| DR 26 Dr. R.E. Moyers                         | 150.00                | 6.94                   |
| DR 27 Dr. J.W. Abbiss and<br>Dr. A.G. Nutlay  | 200.00                | 169.32                 |
| DR 28 Dr. O.J. Walker<br>and Dr. H.R. MacLean | 1000.00               | 915.00                 |
| DR 29 Dr. M.D. Smith                          | 375.00                | -                      |
| DR 30 Dr. N.F. Walker                         | 1400.00               | -                      |
| DR 31 Dr. L.B. Jacques<br>Mr. G.J. Millar     | 1870.00               | 1205.00                |
| DR 32 Dr. A. W. Ham                           | 900.00                | 900.00                 |
| DR 33 Dr. H.A. Hunter                         | 1500.00               | 1500.00                |
| DR 34 Dr. A. D'Iorio                          | 2840.00               | 840.00                 |
| DR 35 Dr. J.B. Macdonald                      | 2000.00               | 1300.00                |

THE CHAIRMAN stated that the figures thus presented indicated that most of the funds at the disposal of the Committee had been allocated but that the second list showed quite a few grantees had not taken up their appropriations and this raised the question as to whether any of the grantees were likely to have an unexpended balance to their credit at 31 March 1951. In this event the funds would be lost to the Committee as all moneys unexpended at the end of the fiscal year revert to consolidated revenues.



It was MOVED by Dr. MacLean and SECONDED by Dr. Thomson,

That the Secretary be directed to write a letter to each grantee pointing out that all the funds at the disposal of the Committee have been allocated and that the Committee still has some applications for grants which it has been unable to award through lack of funds. Each grantee should be asked whether he can foresee having a balance of unexpended funds at 31 March 1951 which might be returned to the Committee by 31 December 1950 and thus made available for re-allocation to a new grantee. Grantees should also be informed that the fiscal year ends 31 March and that a full accounting must be made at that date. If funds are required for two or three additional months (e.g. until the end of the University fiscal year in June) a new application should be made to the Committee at its February meeting.

CARRIED.

DR. RAE said it would be helpful if the Committee year coincided with the University year which ends in June.

DR. HAM added that he thought a good case could be made for more economical expenditures under grants if the Committee's year ended later.

THE CHAIRMAN pointed out that the Committee is bound by the Government's fiscal year which ends 31 March.

It was MOVED by Dr. Thomson and SECONDED by Dr. Rae,

That the Associate Committee on Dental Research feels that its grants-in-aid could be much more efficiently used by workers in universities if it were possible for grants-in-aid to be made for a period corresponding to the university year e.g. from 1 July to 30 June. The present system is felt to be inefficient in several ways; it requires applicants to prepare their cases in February when they have difficulty in foreseeing their plans for the following university session and do not usually know what personnel may be available; it places in an awkward position graduate students and other persons employed on such grants whose salaries now terminate some months before the end of the session; and it is disturbing to grantees, especially to those who hope to continue the project into a further year since it tends to cause an interruption and a period of uncertainty, during which trained personnel may be lost, at the very period of the year when sessional research projects are most likely to be fruitful in results from day to day.

CARRIED.



30. DR 29 Dr. M. Doreen Smith - Additional Grant, \$375 (Min. 36, 11th Mtg.)

THE CHAIRMAN stated that an application from Dr. Smith for \$1500. under this grant for the present fiscal year had been considered and refused at the 11th Meeting of the Committee but that on being advised of this decision Dr. Smith had pointed out that certain work completed by 31 March 1950 resulted in computations and analyses that still remained to be done; she therefore requested that an additional grant be made in the amount of \$375. to cover the employment of Miss Ham for April, May and June (\$350) and \$25. for supplies.

A letter ballot of the Executive approved this application which was now submitted to the Committee for confirmation.

It was MOVED by Dr. Rae and SECONDED by Dr. McDougall,

That the action of the Executive in making a further grant of \$375. to Dr. M.D. Smith for the three months April-June 1950 be confirmed.

CARRIED.

31. DR 1 - Dr. J. J. Rae - Re-allocation of Funds

THE CHAIRMAN read a letter from Dr. J. J. Rae in which it was pointed out that under his grant he was entitled to \$500. for the employment of a full-time assistant during the four summer months. He had, however, been unable to secure a suitable assistant at that salary but his quarter-time assistant, Mr. C. T. Clegg, had severed his connection with his previous employer and was able to devote full time to the project for the three months June-August. Dr. Rae asked permission to pay Mr. Clegg \$100. a month for each of these three months out of the \$500. allotted to him for salaries for the four summer months.

THE CHAIRMAN said that the Executive recommended that this permission be granted. AGREED.

32. DR 30 Dr. Norma Ford Walker (Min. 39, 11th Mtg.)

THE CHAIRMAN pointed out that the original application under DR 30 had been reduced from \$2600 to \$1400 i.e. six months' salaries at \$200. per month and \$200. for supplies.

He said that Dr. Walker had written in July asking that a further grant be made to enable her to employ Dr. W.L. Liu from October 1950 to March 1951 i.e. six months at \$200. or a total of \$1200.

The Executive had considered this renewed request and recommended that it be approved.



It was MOVED by Dr. McDougall and SECONDED by Dr. Ham,

That the recommendation of the Executive be confirmed but that the Chairman be asked to discuss this project with Dr. Walker with a view to its termination as soon as possible. CARRIED.

### 33. Finances (1951-52)

THE CHAIRMAN pointed out that it would shortly be necessary for the Secretary to submit estimates covering the Committee's financial requirements for 1951-52 to the National Research Council. He said there had been a moderate increase in the amount requested for grants and this could be taken as evidence that the Committee had stimulated research in the field of dental sciences. During the present year applications had been received for amounts in excess of the Committee's allotment and several applications had had to be reduced. An increasing number of young men with some graduate training is now becoming available; these men are very keen and are anxious to get projects going. On consideration of all these factors the Executive felt justified in recommending that \$5000. more be provided for grants-in-aid in the coming year but were of the opinion that the other items might be maintained at the same level as in 1950-51. The estimates on this basis would therefore be as follows for 1951-52:

|               |            |
|---------------|------------|
| Grants-in-aid | \$32,500.  |
| Fellowships   | 5,000.     |
| Travel        | 2,500.     |
| Contingencies | 500.       |
|               | <hr/>      |
|               | \$ 40,500. |

It was MOVED by Dr. MacLean and SECONDED by Dr. Rae,

That the Committee recommends to the National Research Council that an amount of \$40,500. be provided for the Associate Committee on Dental Research during the fiscal year 1951-52. CARRIED.

### 34. Membership of the Committee

#### (i) Reappointments

THE SECRETARY reported that the National Research Council had accepted the recommendation of the Committee and had renewed the appointments of five of its members which expired 31 March 1950 for a further term of three years to 31 March 1953. These members are: Dr. C. H. Best, Dr. M.H. Garvin, Dr. H.R. MacLean, Dr. J. J. Rae and Dr. D. L. Thomson.



34. Membership of the Committee (cont'd)

(ii) Successor to Dr. C.B. Stewart (Min. 42, 11th Mtg.)

THE CHAIRMAN reported that the Executive recommended the appointment of Dr. J. B. Macdonald, Assistant Professor of Bacteriology, Faculty of Dentistry, University of Toronto, for the remaining period of Dr. C.B. Stewart's term of office which expires 31 March 1951. AGREED.

(iii) Successor to Dr. H. K. Box

THE CHAIRMAN reported that Dr. H. K. Box had found it necessary to tender his resignation as a member of the Associate Committee on Dental Research because of ill health.

The Executive had considered this matter and had agreed to recommend the appointment of Dr. J.S. Bagnall, Dean, Faculty of Dentistry, Dalhousie University, for the remaining period of the appointment of Dr. Box which expires 31 March 1952.

It was MOVED by Dr. MacLean and SECONDED by Dr. Garvin,

That these two resignations be accepted with regret and that expressions of appreciation for their services be tendered to each of the resigning members and that the recommendations of the Executive regarding their successors be approved and submitted to the National Research Council.

CARRIED.

35. Air Travel

It was MOVED by Dr. Thomson and SECONDED by Dr. Ham,

That permission to travel by air to this meeting be approved for Dr. J.B. Macdonald and Dr.W.J.Linghorne.

CARRIED.

36. Fellowship Applications

THE CHAIRMAN said that he had received on 13 October 1950 through the Chairman of the Research Committee of the Canadian Dental Association, an application for a fellowship which had been made out under date of 13 May, 1950 in the name of Lesley A. Gill. The Executive had considered this application but as it was incomplete the Executive recommended that the Secretary obtain further information and also point out that the deadline for applications is 1 February in each year. Accordingly the application had been shelved for the present.

37. New Research Projects (Min. 44(ii), 11th Mtg.)

THE CHAIRMAN said that at the last meeting of the Committee Dr. Garvin had proposed that subjects for research be prepared. He had mentioned, for example, the very general interest in the problems involved when



gold inlays are made over amalgams. Little information is available as to whether this causes disintegration of the cement. He opened the question for general discussion.

DR. GARVIN said he had a second suggestion to offer namely that work be done on the root canals of teeth. This was a very practical research as the results would be of value to every practitioner.

DR. HAM said that a researcher in this field would have to use animals that have root canals which are similar to those in humans. He thought there were very few places where adequate facilities for such research would be available.

DR. THOMSON said he would speak to Dr. Racey about this matter as he thought Dr. Racey had adequate equipment and might be interested in the problem.

DR. GARVIN said there were many research problems to be found among the subjects on which the profession is not in agreement.

THE CHAIRMAN suggested that all members of the Committee might be invited to furnish a list of proposals on which they are of the opinion that profitable research might be done. This listing could then be included as an appendix to the proceedings for continuous reference.

### 38. News Report of Meeting

THE CHAIRMAN said that in accordance with previous practise the Secretary would prepare a brief report of this meeting for submission to the Journal of the Canadian Dental Association of which Dr. Garvin is the Editor.

### 39. List of Members with Addresses

THE CHAIRMAN said that a request had been received from some of the members for the inclusion of complete addresses of the members of the Committee in each copy of the proceedings and this would be done.

### 40. Date of Next Meeting

#### (i) September 1951

THE CHAIRMAN said that the Canadian Dental Association is holding its national meeting in Ottawa next year 24-25 September and it was AGREED that the Fall meeting of the Associate Committee should be held on the next two following days after the national meeting probably 26-27 September.

#### (ii) March Meeting

It was AGREED that the next meeting of the Committee should be held 1-2 March 1951.

THE MEETING ADJOURNED AT 11 A.M.



## APPENDIX "A"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon.

Grantee: Dr. C. P. Leblond

Project: DR 23 Amount of Grant: \$2,400.

#### SUMMARY OF WORK

Reports on the formation of bones (article No. 1) and teeth (article No. 2) have now appeared in print:

1. Radio-autographic visualization of bone formation in the rat, by C.P. Leblond, G.W. Wilkinson, L.F. Bélanger and J. Robichon. The Am. J. of Anat. Vol. 86, No. 2, March 1950.
2. Mineralization of the growing tooth as shown by radio-phosphorus autographs by L.F. Bélanger and C.P. Leblond. Proc. of the Soc. for Expt. Biol. and Med. 73:390-391. 1950.

The work has continued along two main lines:

- (i) Bones. Since the rat shows only the so-called primary type of ossification, experiments are being carried out in cats and dogs in order to find a species in which the secondary ossification may be conveniently studied with radio-phosphorus.
- (ii) A considerable amount of work has been done on the teeth of hamster rats and mice in the first few days of life. This work has now been extended to older animals with the help of a new machine capable of cutting thin sections of undecalcified teeth. Results obtained so far are similar to those described in the hamster in article No. 2.

A. E. M. Westman  
Signature





APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Investigation of methods for use of methyl methacrylate in direct dental fillings.

Grantee: Dr. A.E.R. Westman

Project

No. DR 25

Amount of Grant: (\$3,000 includes DR 12)

SUMMARY OF WORK

This project was authorized to begin April 1, 1949, and in its first phase dealt with the effect of acrylic fillings on vital teeth. A progress report on this first phase was submitted early in the summer and a detailed report on the research which has been carried out since then is being submitted at this time.

A brief investigation of three commercial brands of direct acrylic filling material has been made. Free volumetric shrinkage has been determined by a dilatometric method and, in view of the rather high values, close attention has been given to shrinkage in general and its possible effects on the suitability of plastics as dental filling materials.

Direct fillings have been placed in extracted teeth and examined for adaptation. An attempt was made to detect leaks by an electrical conductance method. A study has been begun of volumetric shrinkage in confined spaces or cavities and the results of this work promise to reveal some interesting data on the matter of marginal adaptation.

A.E.R. Westman

Signature





## APPENDIX "B"

### ONTARIO RESEARCH FOUNDATION

#### Department of Chemistry

#### Dental Materials Research Fellowship

Project DR-25

Report 5001

This report summarizes an experiment which was begun last year to determine the effect of direct acrylic fillings on vital teeth.

#### General Outline

For this study two young dogs were used. The twelve anterior teeth in each dog were considered the most suitable for the placement of fillings and the subsequent sectioning. Direct acrylic fillings were placed in six of the teeth in each animal using a specially designed heat lamp to cure the fillings. Silicate fillings were placed in the other teeth as controls. A lining of zinc oxyphosphate cement was used in three teeth of each group. The choice of a particular combination of filling materials (e.g. unlined acrylic) for any one tooth (or in other words, the experimental design) was based on random numbers.

After two months the teeth were extracted, mounted in celloidin and sectioned on a microtome. The sections were then examined under a microscope to determine any pathological condition.

#### Materials

The acrylic filling material used in this experiment consisted of methyl methacrylate monomer, from which the hydroquinone inhibitor was removed by washing with alkali and to which was added 3% trihexylamine as a polymerization activator and a commercial grade (Huelon) of acrylic polymer for inlays to which was added 0.5% benzoyl peroxide.

The silicate filling material was Gaulk's Synthetic Porcelain and the cavity lining material was Denco Temporary Cement.

Two young dogs served as experimental animals. One, weighing 9.4 kilograms, was about 6 months old while the other was estimated to be from 9-12 months old and weighed 16 kilograms.

A lamp specially designed for the thermal polymerization of the acrylic fillings was employed. Its use permitted heat to be localized on the area of the filling. It was constructed on the principle of a motion picture projector wherein a lamp, a mirror



and a condensing lens were so arranged as to focus light rays and therefore, heat on a small area. This concentrated beam of energy was then conducted from the lamp to the tooth through a quartz rod. A diagram of the lamp is shown in Figure 13.

### Placement of Fillings

Class V cavities were prepared on the Labio-gingival surfaces of the twelve anterior teeth in each dog and extended into the dentine.

Linings, where used, and silicate fillings were placed in the usual manner without the use of a rubber dam. Silicates were inserted first and protected with cocoa butter while the acrylic fillings were being inserted, cured and trimmed.

The acrylic fillings were made from a mixture of monomer and polymer. A small mound of polymer was moistened with a few drops of monomer on a glass slab. The mixture was stirred briefly and used when it became tacky. Some of the mixture was transferred to the tooth and pressed firmly into the cavity with the fingers, protected by a piece of moistened cellophane. If insufficient resin was placed in the cavity on the first trial, the resin in the cavity was moistened with a small drop of monomer and then more mixture added and compressed. When the filling had become tough (in about 3 to 4 minutes), the pressure was released and the filling was cured with the use of the heat lamp.

Laboratory experiments on extracted human teeth with a thermocouple in the pulp canal had shown that when heat was applied from the lamp for fifteen seconds on the filling and fifteen seconds off and repeated until the filling was satisfactorily cured, the maximum temperature recorded was 120°F. It is believed that this temperature would be lower in vital teeth because circulation would serve to dissipate the heat.

In the practical experiment with dogs, three fillings were packed and then all heat curing carried out at one time so that the heating cycle for one tooth was 15 seconds on and 30 seconds off. After about 6 applications of heat, the fillings were hard enough to be trimmed with a sandpaper disc and polished with whiting and a small felt wheel.

### Experimental Design

The fillings were placed according to the following design, where A, S, L and U represent acrylic, silicate, lined and unlined respectively and the quadrants represent the corresponding areas of the mouth. For example, the notation, /2, would indicate the second tooth from the centre in the upper left quadrant or in other words, the upper left lateral.



| Dog No. 1 |      |      | Age: about 6 months |      |      | Weight: 9.4. Kilos |  |  |
|-----------|------|------|---------------------|------|------|--------------------|--|--|
| S.U.      | A.U. | A.U. | S.U.                | S.U. | A.L. |                    |  |  |
| 3         | 2    | 1    | 1                   | 2    | 3    |                    |  |  |
| 3         | 2    | 1    | 1                   | 2    | 3    |                    |  |  |
| A.U.      | S.L. | A.L. | S.L.                | A.L. | S.U. |                    |  |  |

| Dog. No.2 |      |      | Age: 9.12 months |      |      | Weight: 16 Kilos |  |  |
|-----------|------|------|------------------|------|------|------------------|--|--|
| S.L.      | S.U. | A.L. | S.L.             | A.U. | A.L. |                  |  |  |
| 3         | 2    | 1    | 1                | 2    | 3    |                  |  |  |
| 3         | 2    | 1    | 1                | 2    | 2    |                  |  |  |
| S.U.      | S.L. | S.U. | A.U.             | A.L. | A.U. |                  |  |  |

#### Preparation of the teeth

After the fillings had been in place two months, the animals were sacrificed and their jaws removed and stored immediately in 10% formalin solution. Later the teeth were extracted and prepared for sectioning. This preparation consists, in general, of decalcification in dilute nitric acid, washing, dehydrating in alcohol, saturating with an ether-alcohol mixture and then embedding in colloidion or celloidin. The teeth were then sectioned on a microtome, stained with hemotoxin and eosin and mounted with Canada balsam on microscope slides.

#### Histopathological Study

In the preparation of the teeth of dog No. 2, 8 teeth were so badly damaged by pulp shrinkage that they were worthless for purposes of examination.

Of the 16 remaining teeth, all were vital at the time of extraction, 14 showed evidences of mild hyperemia and 13 had depositions of secondary dentine. The odontoblastic layers were intact, there were no vacuoles and no fibrosis of the pulp. A tabulation of these teeth, with regard to hyperemia and secondary dentine, which were the only evidence of abnormal conditions that were observed, is as follows.



| FILLING | HYPEREMIA |      | SECONDARY DENTINE |        |          |       |
|---------|-----------|------|-------------------|--------|----------|-------|
|         | None      | Mild | None              | Slight | Moderate | Heavy |
| A.U.    | -         | 4    | -                 | 1      | 2        | 1     |
| A.L.    | -         | 5    | 2                 | 1      | 1        | 1     |
| S.U.    | 1         | 2    | 1                 | -      | -        | 2     |
| S.L.    | 1         | 3    | -                 | -      | 2        | 2     |

It is apparent that the zinc phosphate lining material caused mild irritation - 8 of 9 teeth with linings had slight hyperemia and 7 of 9 had depositions of secondary dentine.

The unlined acrylic fillings also caused mild hyperemia and the formation of secondary dentine, although to a degree not greater than the lining material.

It was expected that the teeth with unlined silicate fillings might show devitalization. However, only three of these teeth were suitable for study and of these one was quite normal with no hyperemia or secondary dentine. It is a question whether devitalization might have occurred in these teeth had they been left for a period longer than two months before extraction.

With regard to the use of direct acrylic filling materials, it is evident that mild irritation can be expected probably to the same extent as that caused by zinc phosphate cements. Since the irritation may depend on the cavity depth, it would be wise to use a liner in all deep cavities until further information about this subject is available.

### Summary

An experiment is described wherein dogs' teeth were filled with acrylic filling materials and the results of a histopathological study of the sectioned teeth are reported. Direct acrylic filling materials can be expected to cause mild but not serious irritation to vital teeth and probably to no greater extent than that which is caused by zinc oxyphosphate cements. Protective bases are recommended in all deep cavities until additional experimental evidence to the contrary is presented.

### Appendix

There follow some photographs of decalcified sections of teeth operated on in this experiment. In the low-magnification (20X), photographs, the area outlined by a rectangle is that part of the section which is shown in one other high-magnification (150X) photograph. The solid lines indicate the boundaries of the dentine tubules which lead from the cavity to the area in the high-magnification photograph.



"B-5"

Figures 1, 4, 7 and 10, show 20X photographs of teeth filled respectively by

- (1) acrylic - unlined
- (4) acrylic - lined
- (7) silicate - unlined
- (11) silicate - lined

Figures 2, 5, 8 and 11, show respectively 150X photographs of the areas outlined by the rectangles in Figures 1, 4, 7 and 10.

Figures 3, 6, 9 and 12, are 150X photographs of sections of other teeth filled respectively by the same materials as in 1, 4, 7 and 10.

Figure 1 - 20X. Acrylic unlined

2 12

Figure 2 - 150X. Secondary dentine  
Odontoblasts normal

2 12

(signed:) Douglas R. Christie

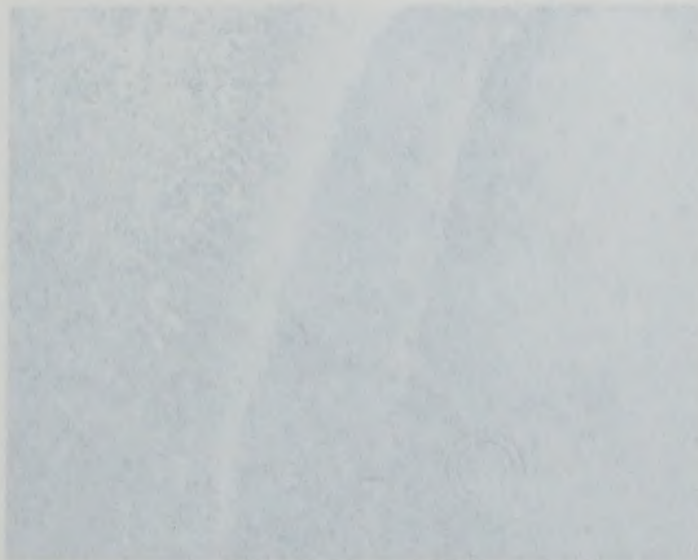


Figure 3 - 150X. Secondary dentine

1 11





"B-6"

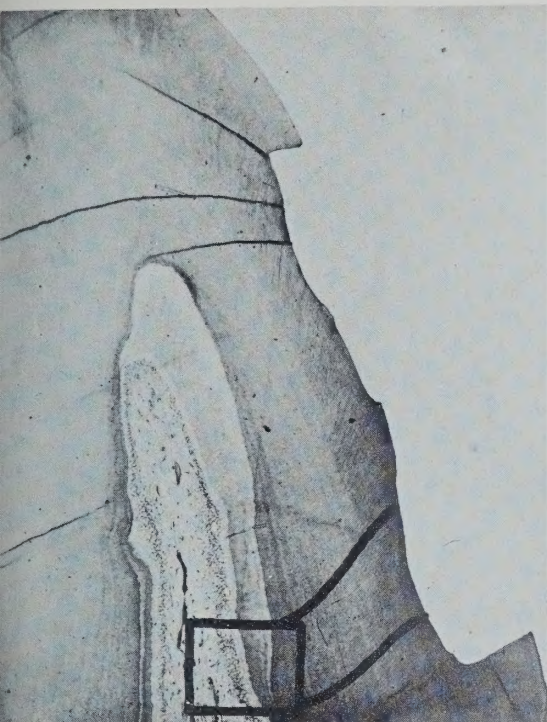


Figure 1 - 20X. Acrylic unlined

2 | 2



Figure 2 - 150X. Secondary dentine  
Odontoblasts normal

2 | 2

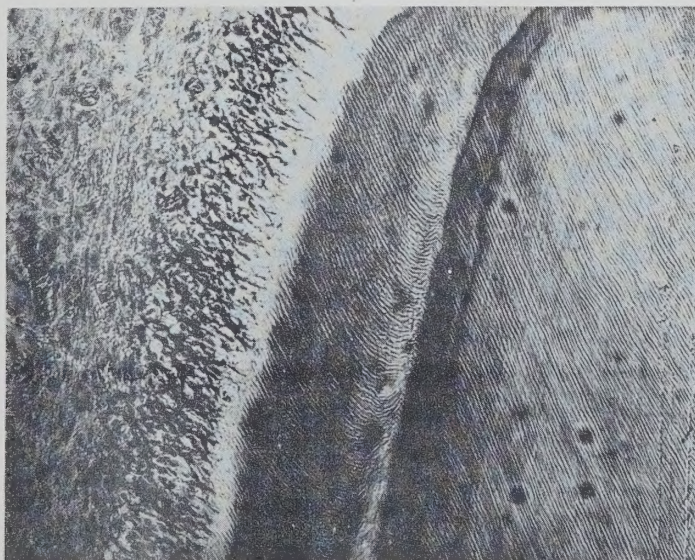


Figure 3 - 150X. Secondary dentine

1 | 1





"B-7"

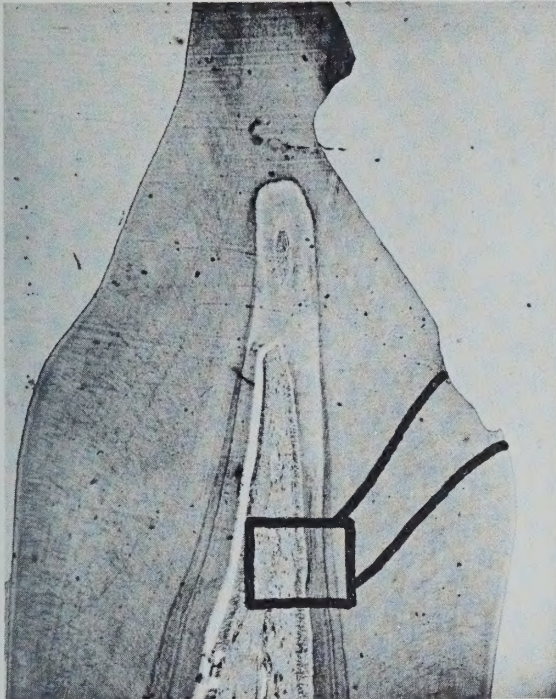


Figure 4 - 20X. Acrylic lined

1 1

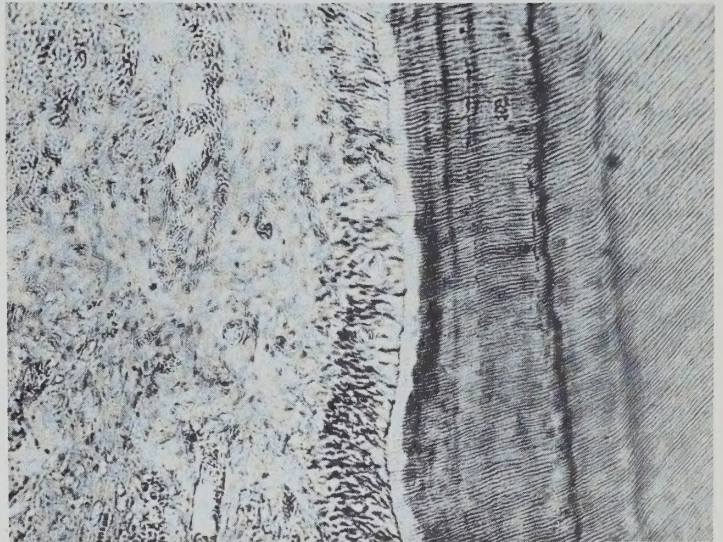


Figure 5 - 150X. Secondary dentine  
Mild hyperemia

1 1

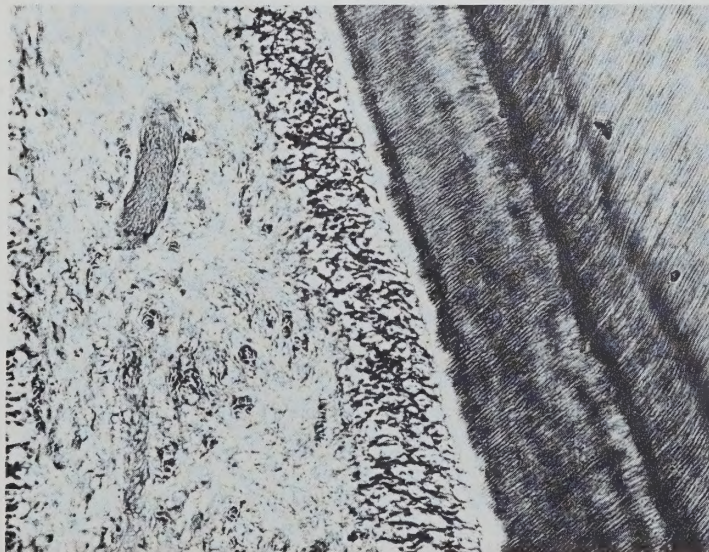


Figure 6 - 150X. Secondary dentine  
Mild hyperemia

1 2





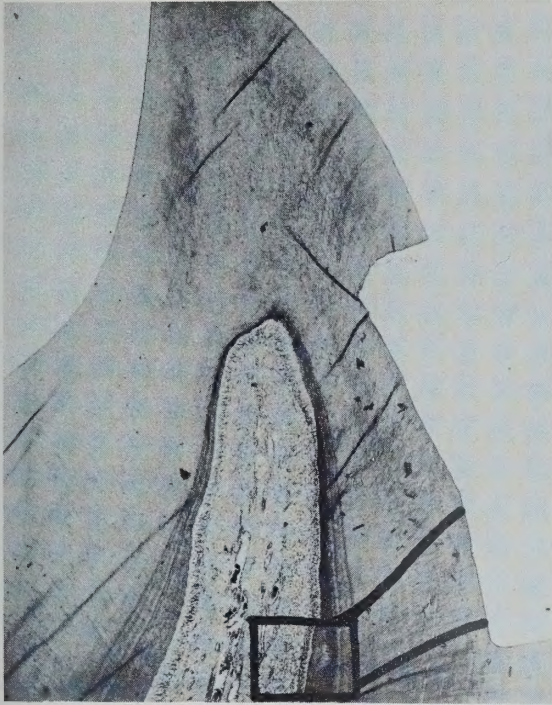


Figure 7 - 20X. Silicate unlined

1 | 1

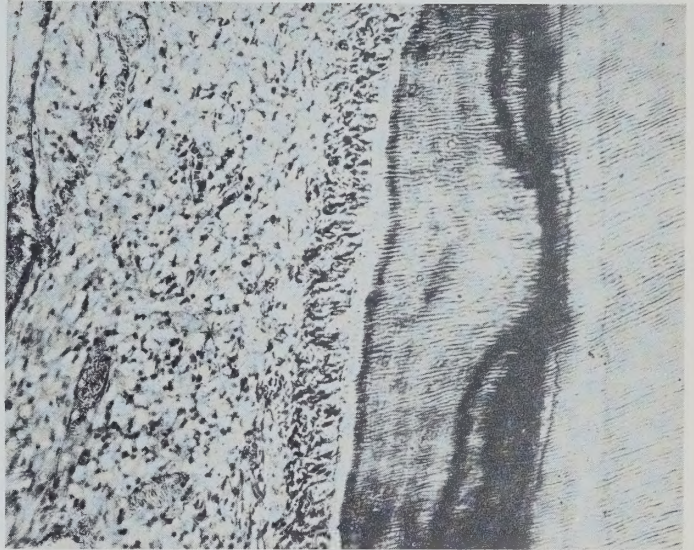


Figure 8 - 150X. Secondary dentine  
Hyperemia

1 | 1

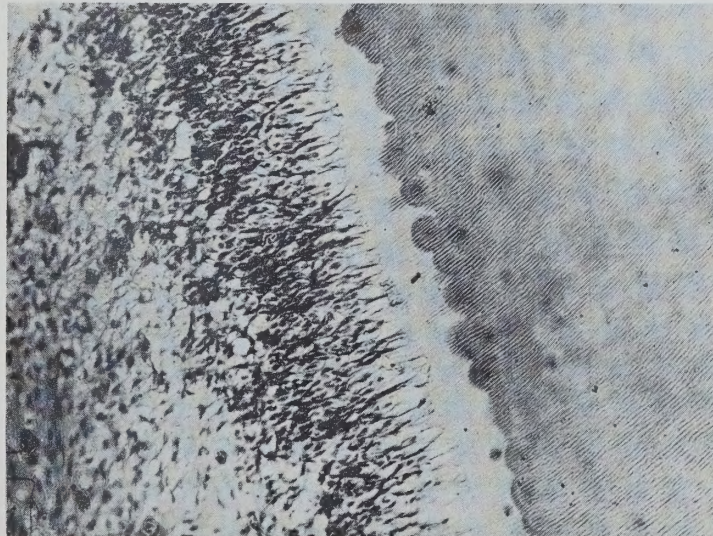


Figure 9 - 150X. No secondary dentine

1 | 2





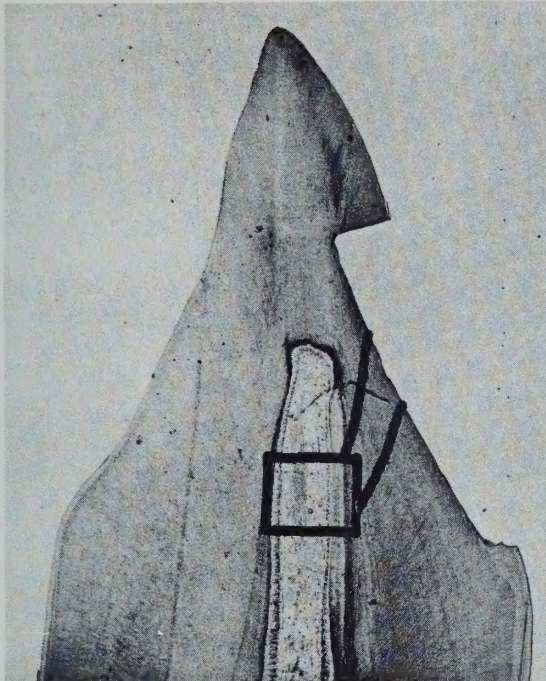


Figure 10 - 20X. Silicate lined

1 | 1

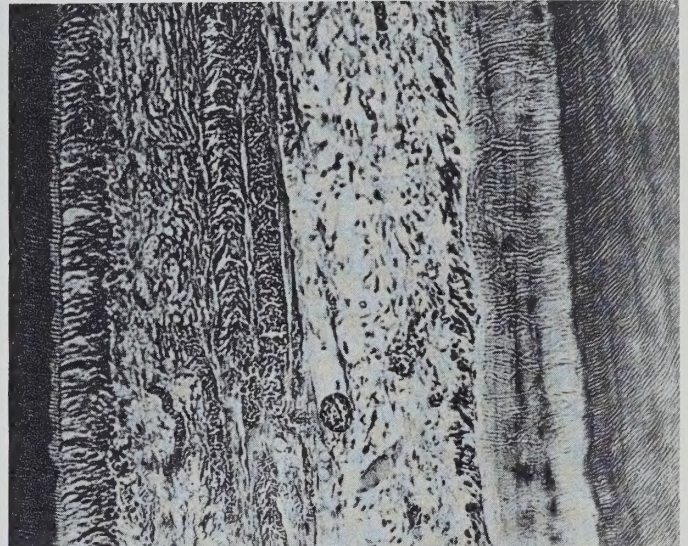


Figure 11 - 150X. Secondary dentine  
Hyperemia

1 | 1

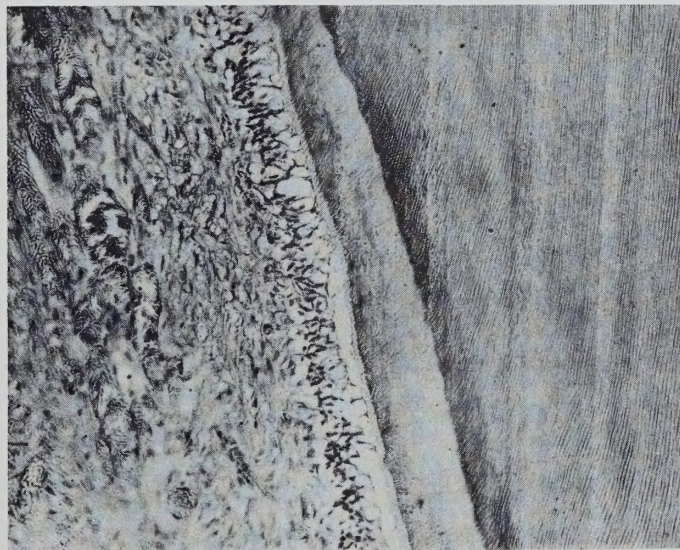


Figure 12 - 150X. Secondary dentine  
Hyperemia

1 | 2





DETAILED RESEARCH FUNDATION

Department of Chemistry

Dental Materials Research Fellowship

Report 3002 - 24 25

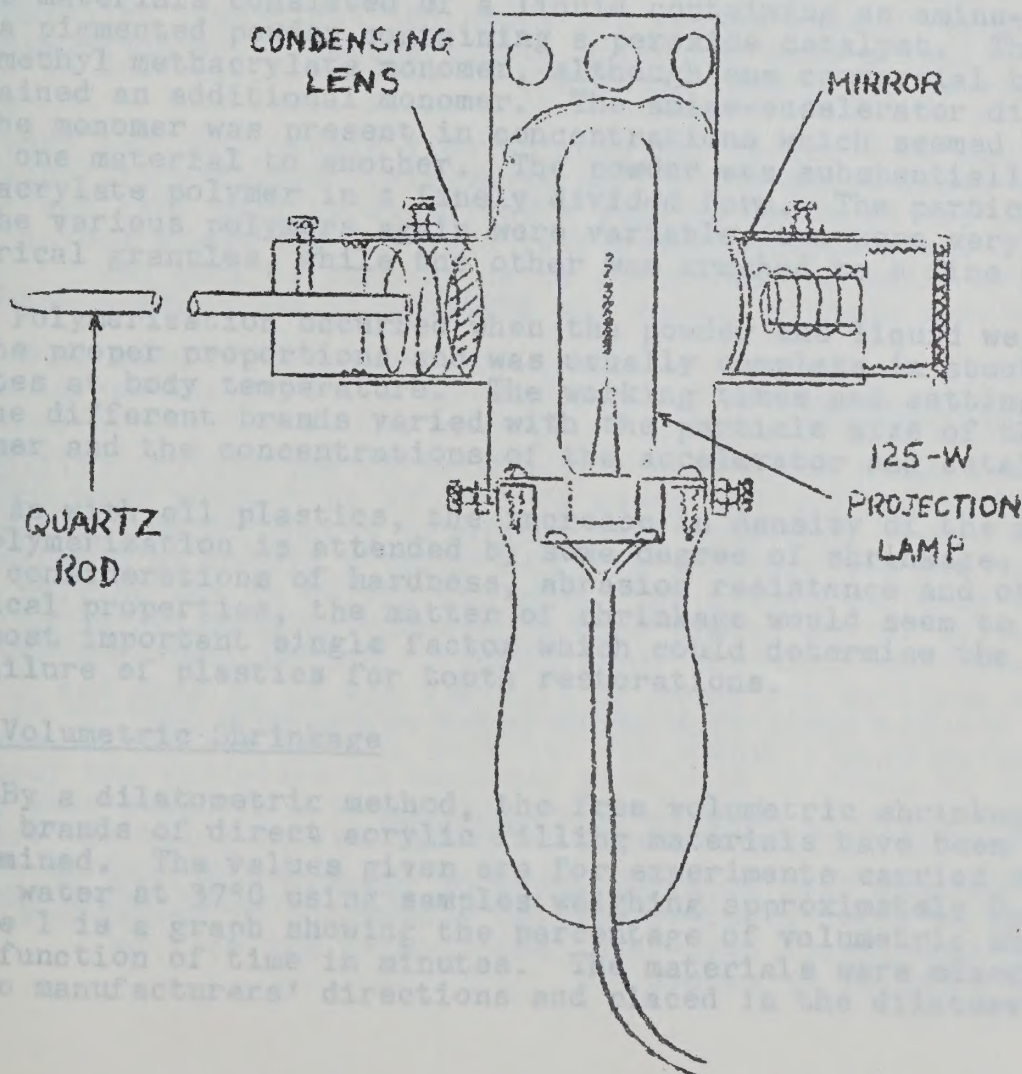
This report is concerned with experimental investigations of methyl methacrylate filling materials. The report on the effect of direct acrylic fillings on the strength of dogs was submitted early in the summer and can probably be found in the literature. The committee

FIGURE 13

DIAGRAM OF HEAT LAMP USED TO CURE DIRECT ACRYLIC FILLINGS

Direct Acrylic Filling Materials

Early this year several commercial brands of direct acrylic filling materials became available for use in operative dentistry. We have investigated briefly three of these products. Essentially these materials consisted of a liquid monomer, an initiator, and a pigmented filler. The liquid was methyl methacrylate. The initiator was benzoyl peroxide. The filler was a finely ground acrylic polymer. The liquid contained an additional component which seemed to vary from one material to another. This component was a methyl methacrylate polymer in the form of very fine spherical granules. The particle size of the various polymers varied from 0.1 to 0.5 microns. The granules were added to the monomer in the proper proportions. The materials were cured in the proper proportions. The curing time was about 1-2 minutes at a body temperature. The curing time of the different brands varied with the particle size of the polymer and the concentrations of the various components.



on polyethylene is attended by a loss of mechanical strength. Apart from considerations of hardness, resistance to wear, and other physical properties, the matter of shrinkage would seem to be the most important single factor which could determine the success or failure of plastics for tooth restorations.

Free Volumetric Shrinkage

By a dilatometric method, the free volumetric shrinkages of three brands of direct acrylic filling materials have been determined. The values given are for experiments carried out under water at 37°C using samples of approximately 0.5 grams. Figure 1 is a graph showing the percentage of volumetric shrinkage as a function of time in minutes. The materials were mixed according to manufacturers' directions and placed in the dilatometer.





## APPENDIX "B"

### ONTARIO RESEARCH FOUNDATION

#### Department of Chemistry

#### Dental Materials Research Fellowship Report 5002 - DR 25

This report is concerned with experimental investigations of methyl methacrylate filling materials. A report on the effect of direct acrylic fillings on the vital teeth of dogs was submitted early in the summer and can probably be found as an appendix of the committee's proceedings.

#### Direct Acrylic Filling Materials

Early this year several commercial brands, of direct acrylic filling materials became available for use in operative dentistry. We have investigated briefly three of these products. Essentially these materials consisted of a liquid containing an amine-accelerator and a pigmented powder containing a peroxide catalyst. The liquid was methyl methacrylate monomer, although one commercial brand contained an additional monomer. The amine-accelerator dissolved in the monomer was present in concentrations which seemed to vary from one material to another. The powder was substantially methyl methacrylate polymer in a finely divided form. The particle sizes of the various polymers again were variable; two were very fine spherical granules, while the other was crushed to a fine dust.

Polymerization occurred when the powder and liquid were mixed in the proper proportions and was usually complete in about 15-20 minutes at body temperature. The working times and setting times of the different brands varied with the particle size of the polymer and the concentrations of the accelerator and catalyst.

As with all plastics, the increase in density of the monomer on polymerization is attended by some degree of shrinkage. Apart from considerations of hardness, abrasion resistance and other physical properties, the matter of shrinkage would seem to be the most important single factor which could determine the success or failure of plastics for tooth restorations.

#### Free Volumetric Shrinkage

By a dilatometric method, the free volumetric shrinkages of three brands of direct acrylic filling materials have been determined. The values given are for experiments carried out under water at 37°C using samples weighing approximately 0.8 grams. Figure 1 is a graph showing the percentage of volumetric shrinkage as a function of time in minutes. The materials were mixed according to manufacturers' directions and placed in the dilatometer



as soon as they reached a dough-like or working consistency. The time, then, at the beginning of each curve is approximately an indication of the working time at room temperature. It appears to be proportional to the particle size of the polymer powder. Kadon, for example, had the finest polymer and had the shortest working time.

The curves show at what point the most rapid and most extensive polymerization occurred. For Kadon this came between 4 and 5 minutes after mixing; for Replica, about 6 to 8 minutes and for Fastcrown between 7 and 9 minutes. After 20 minutes the materials had decreased in volume by the following amounts:

|           |      |
|-----------|------|
| Kadon     | 5.9% |
| Fastcrown | 4.8% |
| Replica   | 4.6% |

Figure 1 also illustrates the effect of the amount of monomer on the shrinkage. Three curves are shown for Fastcrown, representing mixes of thin, medium and thick consistencies. The shrinkages were respectively 4.3%, 4.8% and 5.8%.

The values we obtained are considerably higher than the reported values of other types of filling materials. Silicate fillings, for instance, are reported to have a shrinkage of less than one per cent. It would appear, therefore, that the shrinkage factor should receive close investigation in an attempt to show how these dimensional changes affect the suitability of plastics for fillings.

#### Microscopic Examination

Extracted human teeth were filled with direct acrylic fillings and then conditioned for two weeks at 37°C in an aqueous solution of methylene blue. They were then carefully ground under a stream of cold water and the sections examined under a binocular microscope. All teeth so examined showed very good adaptation of the acrylic filling material to the cavity (and apparently no penetration of the dye between the tooth and the filling). Microphotographs of two of these sections are shown in Figures 2 and 3. On each has been marked a solid black line whose width represents one thousandth of an inch (.001").

#### An Attempt to Determine Leakage by Conduction

It was thought that marginal leaks of plastic fillings if present might be shown by an electrical conduction or resistance method. Our experiments have been inconclusive but will be described as a matter of record.



Figure 4 is a cross-section of the experimental arrangement of the tooth. An extracted tooth was prepared by first drilling a cavity for the acrylic filling, then drilling and excavating the pulp canal to a point behind the cavity. The pulp canal was then packed with dental amalgam and a copper wire lead was embedded in it. The root end of the tooth was covered with sticky wax for insulation. The floor of the cavity was extended to the amalgam in the pulp canal and this sub-cavity also filled with amalgam. The acrylic filling was inserted, the tooth was mounted through a cork stopper close to an external amalgam electrode also containing a copper wire lead and placed in a test tube containing an aqueous electrolyte. The electrical resistance of the system was measured by means of a Triplett meter. It was expected that a leak at the margin would cause a sharp drop in resistance.

It was found that resistances frequently fell off sharply and appeared to reach a limiting value. Later it was discovered that the moisture content of the tooth was an important factor and that the resistance drops which we measured could have been caused by the water absorption. The absorption of water by methyl methacrylate did not, in our experiments, cause any decrease in resistance. Some trouble was also encountered with the electrolyte which at first was a 5% solution of sodium chloride. Its use caused the rapid formation of insoluble salts on the external electrode and led to the formation of a battery effect. This was overcome by using a 5% solution of potassium nitrate. Consideration was given to insulating the tooth against moisture absorption but the method seemed to have doubtful value and was discontinued.

### Volumetric Shrinkage in Cavities

When some fillings were being placed in extracted teeth, it was observed that, if the exposed surface of the acrylic filling was closely adapted to the contour of the tooth by a matrix band, a concavity frequently developed on this exposed surface which was not in keeping with the contour. Obviously the free surface of the filling undergoes considerable shrinkage. It is recommended that when a matrix band must be used every attempt should be made to build up an excess of resin which can later be trimmed to the contour of the tooth. Where possible, the use of a compound matrix which will form an oversize filling is recommended.

In another experiment also excessive shrinkage of the free surface of acrylic filling material was noted. We were proposing to study the variation in hardness with bulk of resin by polymerizing some resin in a wedge-shaped steel mold. The exposed surface was being molded against a flat glass plate so that a smooth surface would be produced for testing purposes. This exposed area, however, invariably pulled away from the glass



leaving a concave, relatively rough surface. The remainder of the resin appeared to adhere tightly to the mold surfaces and the wedge of resin had to be broken to be removed. (Incidentally no appreciable difference in hardness was detected between the material at the shallow end of the wedge and that at the deep end of the wedge and the matter was not pursued beyond the testing of two specimens.)

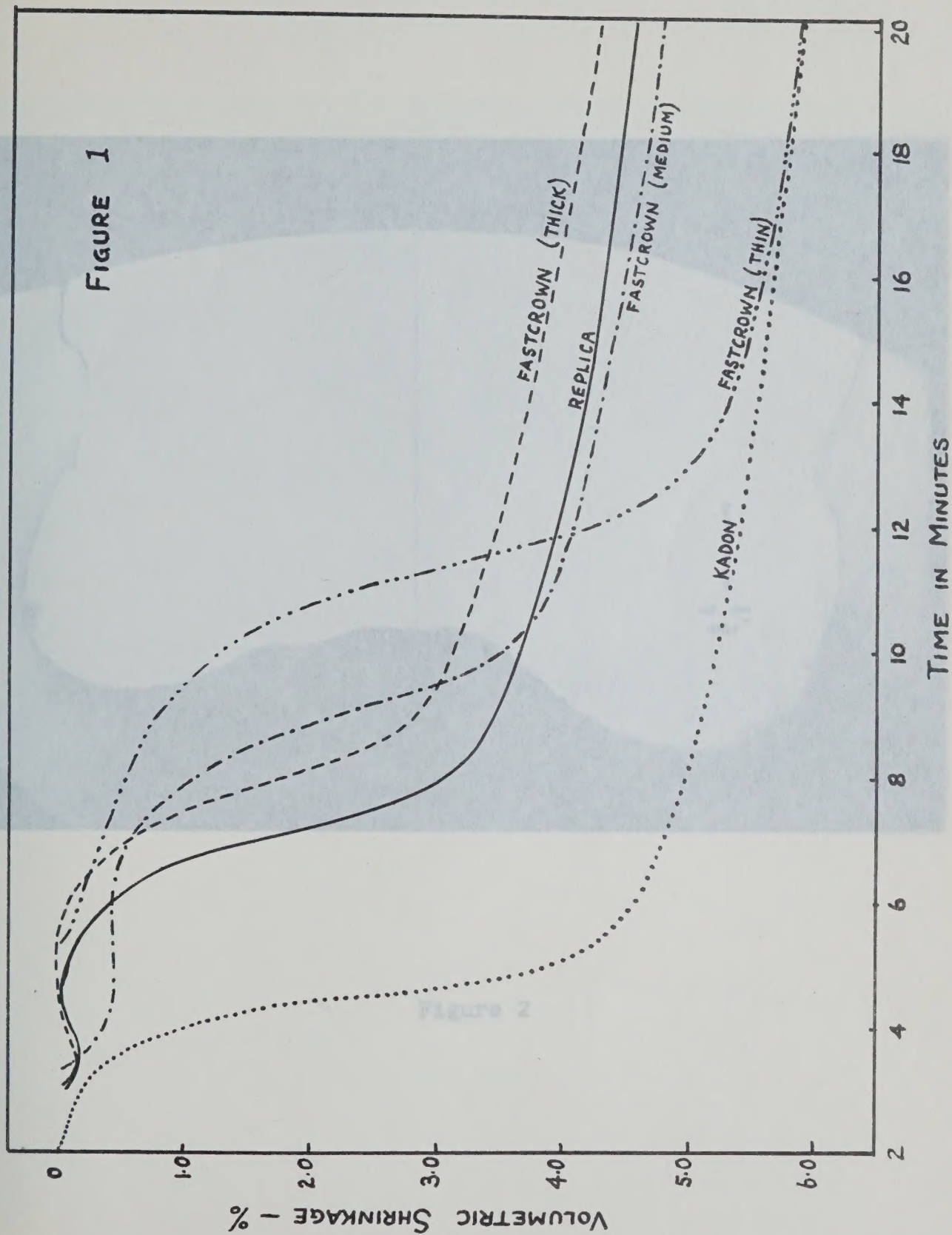
We are now investigating the matter of the volumetric shrinkages of direct acrylic filling materials in closed cavities in an endeavour to determine what may be expected of them under these circumstances. The work has not proceeded far enough at this time to enable us to make any definite statements but a comparison of volumetric shrinkage in confined spaces and free volumetric shrinkage has revealed some interesting data. This information promises to have an important relation to marginal adaptation. Progress reports will be submitted at intervals as has been our custom in the past.

Douglas R. Christie

19 Sept. 1950.



FIGURE 1







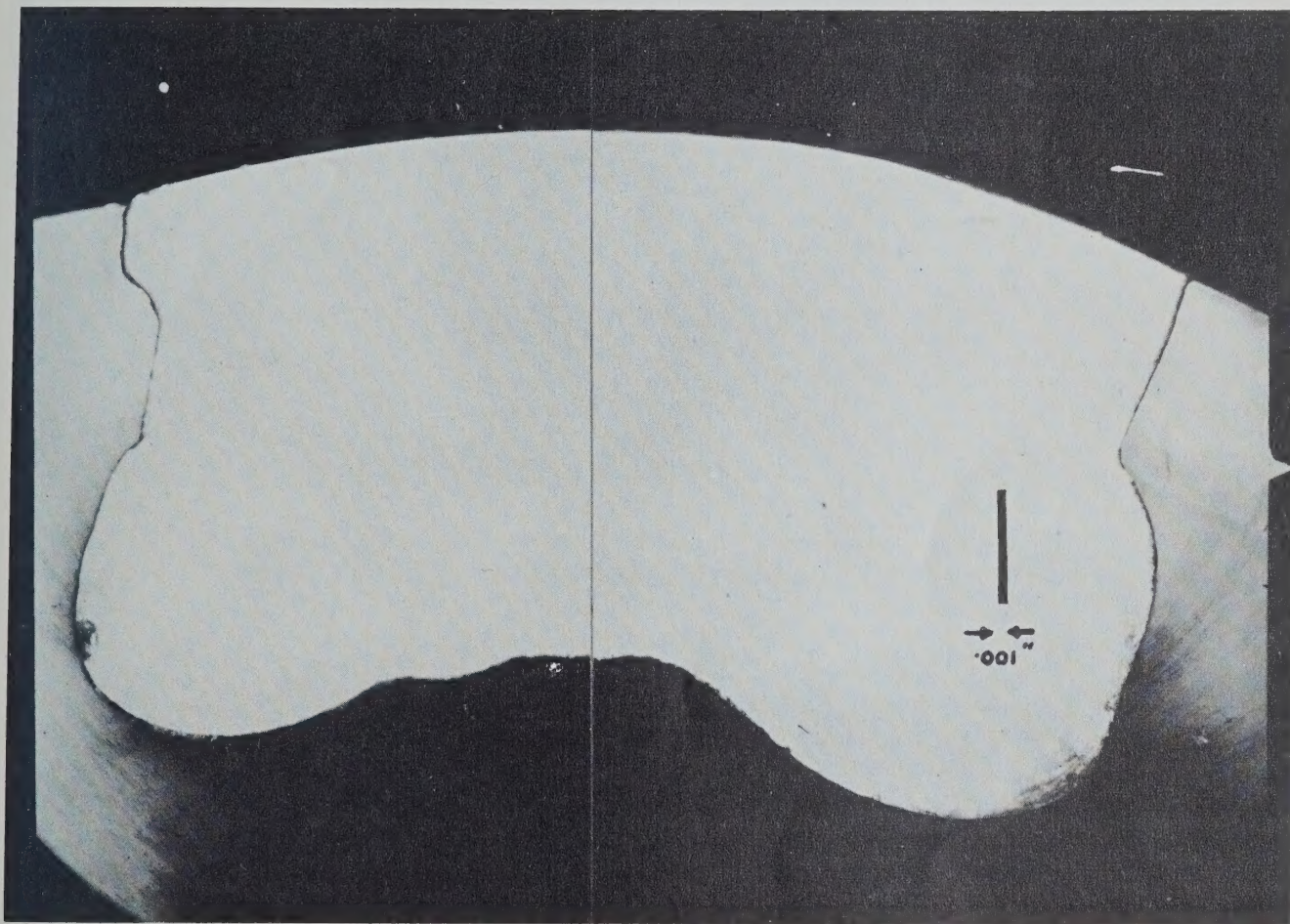


Figure 2





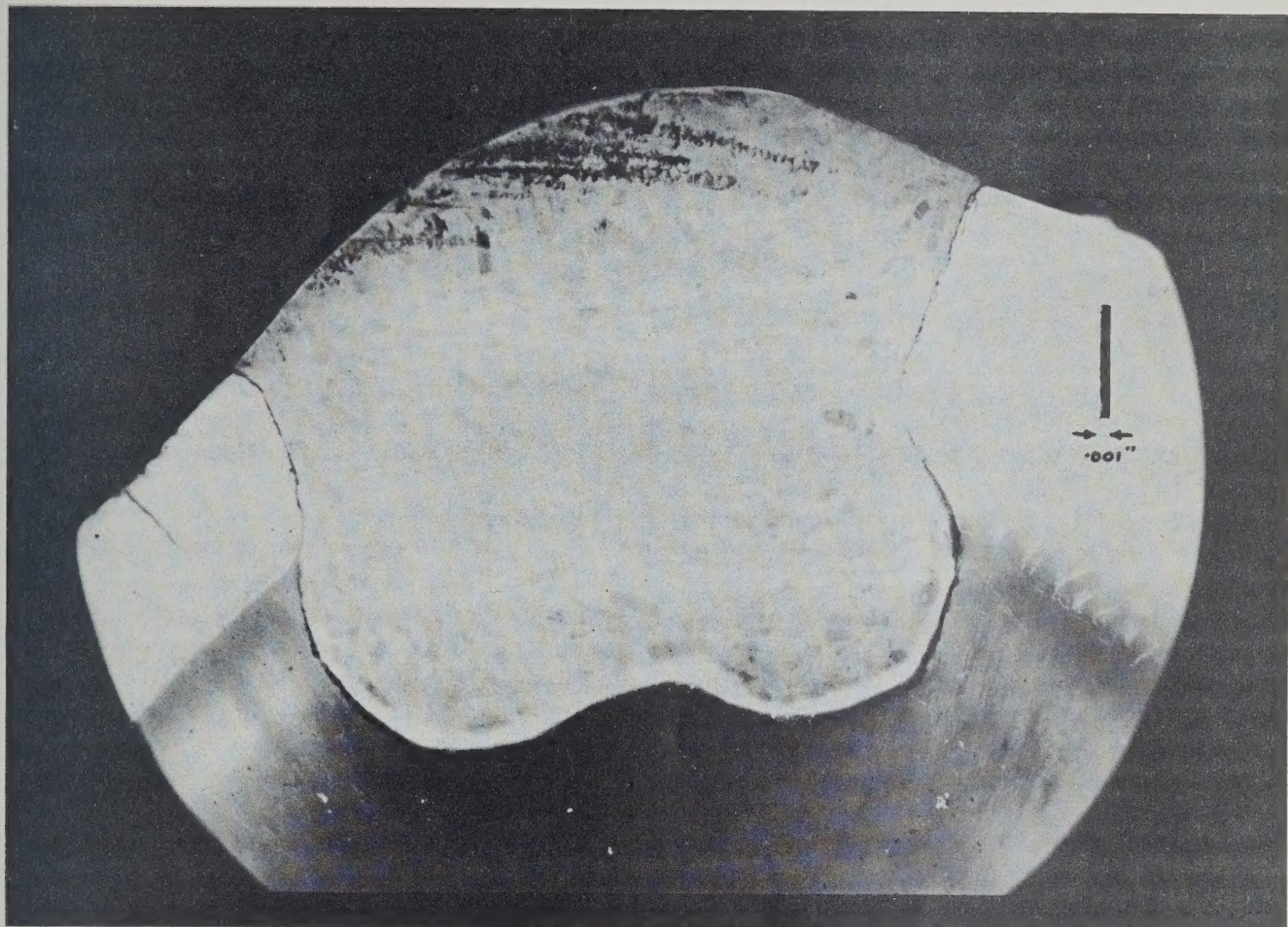


Figure 3





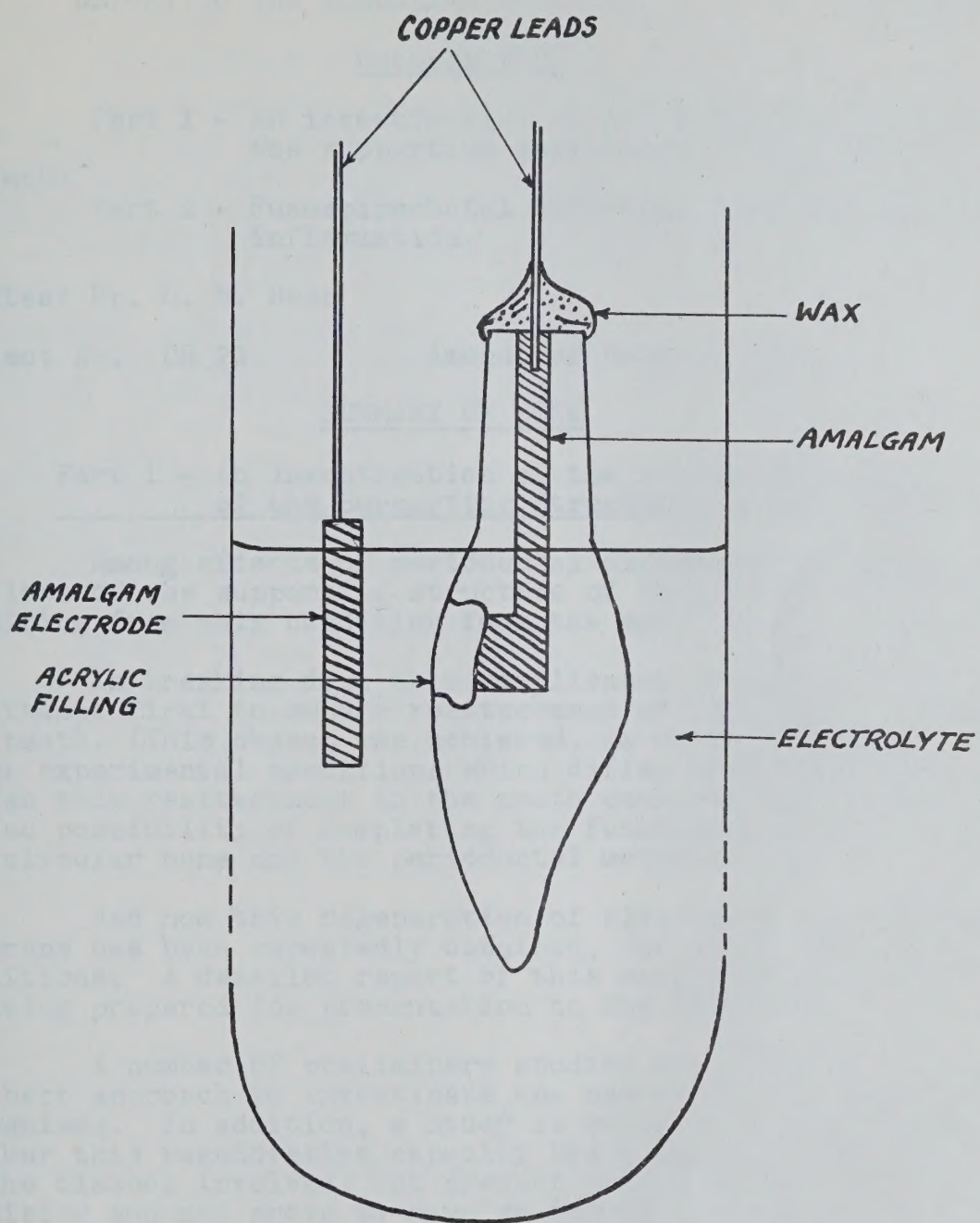


FIGURE 4





APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1950

Subject: Part 1 - An investigation of the mechanism of repair of the supporting structures of the teeth;  
Part 2 - Fusospirochetal infection and pre-existing inflammation.

Grantee: Dr. C. H. Best

Project No. DR 22 Amount of Grant: \$3,800

SUMMARY OF WORK

Part 1 - An Investigation of the Mechanism of Repair of the Supporting Structures of the Teeth

Among effects of periodontal disease are the detachment and loss of the supporting structure of the teeth. The problems resulting from this condition form the main object of this study.

In breaking down this complicated problem, it was decided to attempt first to secure reattachment of the connective tissue to the tooth. (This object was achieved, as we have reported, but only under experimental conditions which differ from clinical conditions.) Unless this reattachment to the tooth could be accomplished, there was no possibility of completing the functional unit by regenerating the alveolar bone and the periodontal membrane.

And now this regeneration of alveolar bone and periodontal membrane has been repeatedly obtained, but still under experimental conditions. A detailed report of this second phase of the study is being prepared for presentation to the committee.

A number of preliminary studies are under way to determine the best approach to investigate the nature of the regenerative mechanisms. In addition, a study is being made to determine whether this regenerative capacity has a place in the physiology of the tissues involved. At present this study is the most promising and may prove to have an important application in the treatment of periodontal disease. However, more work is needed before definite conclusions can be drawn.

W. J. Linghorne

SIGNATURE



SUMMARY OF WORK

Part 2 - Fusospirochetal Infection and Pre-existing Inflammation

Experimental fusospirochetal infection of laboratory animals by the injection of gingival scrapings from mouths with periodontal disease is being studied. Factors influencing infection are being investigated. Although this work is not complete, progress has been made.

A bank of infective exudates from experimental fusospirochetal lesions in guinea-pigs has been established for use in this project. These exudates are preserved in a dry-ice chest and are available at all times.

A complete study of experimental fusospirochetal infection in normal animals was necessary before experiments concerning unknown factors could be set up. This first phase of the investigation has been completed and the second phase is in progress.

A new approach to the investigation of experimental fusospirochetal disease is being made through the use of the rat. These studies are not complete but some facts have been learned about the susceptibility of the normal and abnormal rat to experimental infection with fusospirochetal mixtures. New work using the rabbit has also been done giving favourable results which may be valuable in future serological and immunological studies in relation to fusospirochetal infections.

D.C. O'Connell

SIGNATURE

W. J. Lippincott  
SIGNATURE



REPORT ON - Part 2 - Fusospirochetal Infection and Pre-existing Inflammation

Experimental fusospirochetal infection of guinea-pigs, rats and rabbits by means of the subcutaneous and intra-dermal injection of materials containing the fusospirochetal complex of organisms has been studied and some of the factors influencing these infections have been investigated. Although the project is not complete, many facts which have a definite effect upon experimental fusospirochetal infection have been learned.

One hundred and fifty guinea-pigs used in this investigation, in which experimental disease has been produced, have served as a source of fusospirochetal exudate with which a bank of infective material has been established. These exudates have been preserved and are readily available for use at any time. The infection of guinea-pigs and the transmission of infection from animal to animal has been followed. The type of material required to induce initial infection, the necessity of trauma to effect a lesion, the dilution of fresh material and its bearing on infection, and the examination by dark ground illumination of the fusospirochetal organisms in the exudates have been considered.

The use of the white rat as an experimental animal in this type of investigation has been studied. About 90 animals have been used to compare susceptibility to fusospirochetal infection in the white rat to that in the guinea-pig. The susceptibility to infection with both fresh exudate and transmitted infective material has been investigated in the normal rat, the vitamin B and choline deficient rat and the alloxan diabetic rat. The white rat, being susceptible to experimental infection with fusospirochetal organisms, may prove to be more useful in this project than the guinea-pig. Being a more omnivorous animal, it more readily lends itself for use in studies involving feeding experiments. An attempt will be made to study some of the factors influencing experimental fusospirochetal infection by the use of the white rat. Blood dyscrasias, avitaminoses, nutrition, endocrine dysfunction allergy and mineral metabolism are some factors thought to influence infection in periodontal disease and they can be studied in the rat. Before these subjects can be investigated, it is necessary to determine the minimum infecting dose for the rat of fusospirochetal material from disease processes.

The rabbit has not been used to a great degree in experimental fusospirochetal infection studies because when injected subcutaneously it so readily succumbs to chance infection with streptococci and other pathogens. However, in our investigation it was possible to study the effect of intra-dermal injection of fusospirochetal exudates. Typical abscesses containing large numbers of fusospirochetal organisms have been induced in the rabbit by the injection of quite small amounts (0.1, 0.05cc) of infective exudate from experimental lesions in the guinea-pig.



This part of the problem involved only twelve animals and is by no means complete. A more comprehensive study of this type of infection in the rabbit is planned and the results, it is hoped, will be useful in future experiments in an investigation of the serology and immunology of experimental fusospirochetal infection.

Most of the preliminary experiments in this investigation have been completed but several parts of the experiments must be repeated for confirmation of some points. When this work is accomplished, the originally-planned studies can be carried out.

September 11, 1950.

D. C. O'Connell  
for C. H. Best



APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals.

Grantee: Dr. A. W. Ham

Project No. DR 32

Amount of Grant: \$900.

SUMMARY OF WORK

It was not found practicable to begin this work until this Fall (1950), and hence there is no report available at this time.

It has been hypothesized that growth factors not present in the media may be elaborated by the animal growth. In the theory that such growth factors if present might be distinguishable as a component was performed in which a solid medium streaked with fusospirochetal suspension was separated from a heavy growth of the total flora by a dialysis membrane. Tested with three different fusospirochetal suspensions this method could not be shown to promote growth of individual members of the suspensions.

A. W. Ham  
\_\_\_\_\_  
Signature

13 Sept. 1950.





APPENDIX "E"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Experimental fusospirochetal infection with mixtures of pure cultures.

Grantee: Dr. J. B. Macdonald

Project

No.: DR 35

Amount of Grant: \$2,000

SUMMARY OF WORK

A suitable guinea pig fusospirochetal exudate has been developed by serial passage through guinea pigs of several different suspensions of fusospirochetal organisms obtained from patients with periodontal disease.

Preliminary attempts at isolation of pure cultures from the guinea pig exudate chosen as most suitable has yielded 14 pure cultures of cocci and anaerobic non-motile rods. This represents only a fraction of the organisms known to be present.

Efforts have been made to develop new media and methods for the growth and isolation of the anaerobes characteristic of these infections. Several media have been compared by testing their ability to support the growth of the total flora of 10 different fusospirochetal suspensions. Blood agar prepared with a veal heart infusion (Proske Sayers) base has been found to be slightly more reliable than the other media tried.

It has been hypothesized that growth factors not present in the media may be elaborated by the mixed growth. On the theory that such growth factors if present might be dializable an experiment was performed in which a solid medium streaked with fusospirochetal suspension was separated from a heavy growth of the total flora by a dialysis membrane. Tested with three different fusospirochetal suspensions this method could not be shown to promote growth of individual members of the suspensions.

John B. Macdonald

Signature.





## APPENDIX "E"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Experimental fusospirochetal infection with mixtures of pure cultures.

Project No. DR 35

Grantee: Dr. J. B. Macdonald

Several experiments are being conducted simultaneously, but for clarity will be reported consecutively.

#### 1. Development of a stabilized guinea-pig fusospirochetal exudate

Five patients with various forms of periodontal disease were used as sources of fusospirochetal organisms. Material from gingival crevices and periodontal pockets was collected with sterile scalers and suspended in 1c.c. of Hartley broth. After examination under dark field, the suspension was divided into equal parts and inoculated with trauma subcutaneously into the groin of two young adult guinea pigs. The resultant lesions were examined daily, and sometime before the eighth day, when available, exudate was obtained from one of the animals. Exudate thus obtained was examined by dark field microscopy, and inoculated in 1/10 c.c. doses into two more guinea pigs. It was also streaked on an aerobic heart infusion blood agar plate. This procedure was repeated through several passages in an effort to obtain a typical fusospirochetal exudate in which non-essential organisms had been eliminated by body defenses of the animal.

The results of this experiment are indicated in Table I. It will be seen that in 4 of 5 cases the lesions and dark field appearance of the exudate were more or less typical although there was some variation in the severity of the lesions. Even from the first passage the aerobic flora was relatively scant - not more than two types of the great variety of aerobic bacteria uniformly present in gingival scrapings surviving.

The exudate designated "HG" was chosen as the most suitable for the recombination experiment.

#### 2. Isolation of pure cultures from HG

Preliminary attempts at isolation of pure cultures were made starting with the fourth guinea pig passage exudate of HG. This exudate was streaked on several different media, was inoculated into the wells of spirochete cup plates, and was inoculated into 2 guinea pigs to verify the maintenance of pathogenicity.



The cultures were incubated in Brewer's anaerobic jars from which the oxygen had been removed by combination with hydrogen catalyzed by heated platinumized asbestos and into which CO<sub>2</sub> had been introduced to a concentration of 5%. Incubation period varied from 4 to 9 days at 37°.

When the jars were opened colonies were examined under a 10 power colonial microscope. Dark fields were prepared from the various colonies, and each colony type was subcultured to one of several fluid media and in some cases to fresh plates. After the sub-cultures were incubated anaerobically they were examined by Gram stain, checked for purity, and frozen and stored in the CO<sub>2</sub> ice chest.

It was previously shown that the total flora grown as a mixture in spirochete cup plates maintained its pathogenicity for guinea pigs through 10 culture passages. For this reason the growth obtained from a spirochete cup plate was emulsified with 2.5 c.c. Hartley broth in a tissue grinder and treated in the same way as the original guinea pig exudate. This procedure was repeated through 4 culture passages according to the scheme shown in Figure 1.

The spirochete cup plates have been previously described. They are specially made Petri dishes, the inner member measuring 25 mm X 28 mm deep. The media used in the cup plates consisted of Difco heart infusion base with 1% agar to which was added sterilely 30% Seitz-filtered ascitic fluid, 0.1% neutralized cysteine HCl, and 0.5% of kidney extract prepared as a 10% solution. Since the preparation involved a number of sterile operations, including dispensing of the medium into the cup plates, it was performed in a closed hood with a glass front, equipped with electric outlets, gas, and compressed air, and a suction apparatus by which pipettes could be operated. While in use the hood was fed air filtered through a flask of sterile gauze.

The kidney extract was prepared from the kidneys of a guinea pig of about 400 gms. The kidneys were removed sterilely, minced, and homogenized with sterile distilled water in a Waring blender.

Media which were used for streaking were:

1. Difco heart infusion medium with 2% agar to which was added 10% defibrinated sheep blood.

2. Heart infusion - ascitic fluid - cysteine - kidney extract agar (HIAFKE), the same medium as used in the cup plates, prepared as either 1% or 1.5% agar.



3. Heart infusion - ascitic fluid - cysteine - gentian violet agar, the same medium without kidney extract but containing 0.2% dextrose and 0.003% gentian violet.

Fluid media were:

1. Hartley digest broth.
2. Hartley digest broth plus ascitic fluid 20%.
3. HIAFKE Fluid medium.
4. Thioglycollate medium prepared by Connaught Laboratories and dispensed in screw-cap bottles.

Results:- A total of 42 colony types were subcultured. Of these only 14 survived, and these were cocci and non-motile rods. This represents only a fraction of the organisms present. Failure to isolate more pure cultures is attributed largely to inadequacy of the fluid media, particularly the thioglycollate obtained from Connaught which did not compare well with the previous thioglycollate media which have been used in this work.

It will be necessary to prepare improved media and repeat this experiment.

From the plates streaked from the third culture passage, spirochetes were found growing as isolated colonies into the blood agar. This is a very rare occurrence and the reason why it suddenly occurred is not known. Subcultures from spirochete colonies to fresh blood agar, HIAFKE, agar, and spirochete cup plates all failed to survive.

### 3. Studies of media and methods for isolation of pure cultures

a. It has been recognized that vibrios and perhaps spirochetes seem to be the most difficult members of the whole fusospirochetal group to grow, and that methods used to date are at best marginal and are probably often inadequate. Efforts have therefore been made to learn something of the growth characteristics of vibrios by attempting to isolate them from various media by two different methods. First, exudates were streaked in the conventional way and colonies resulting were examined by dark field. Secondly, because vibrios are motile, drops of exudate were placed on various media in the hope that vibrios might migrate beyond the edge of the drop. All cultures were incubated anaerobically for 5 days.

Results:- Using 4 different media and a total of 20 different exudates in repeated trials, no vibrios were isolated from the streak plates. Nor were vibrios isolated from the confluent drops, but dark field examination of the growth of confluent drops showed the presence of vibrios in many instances.



It was felt that examination under dark field of the growth of confluent drops might be a useful method of screening media i.e. growth of vibrios in isolated colonies could not be expected where the organisms failed to grow in mixed culture.

b. To test various media for their ability to support growth of vibrios and other organisms in mixed culture, ten different fusospirochetal suspensions were inoculated as confluent drops on 5 different media and into the wells of HIAFKE spirochete cup plates. Six of the suspensions were exudates obtained from guinea pigs, three were broth suspensions of gingival scrapings, and one was mixed growth from a cup plate emulsified in broth. The inoculated media were incubated in anaerobic jars for 6 days and then cross sections of the confluent growth were examined by dark-field illumination. Table 2 summarizes the results for spirochetes, vibrios and filaments. In addition, the occurrence of spirilla, fusiforms, non-motile rods and cocci was recorded. In the case of spirilla the occurrence was too sporadic to be of significance. Fusiforms occurred 8 or 9 times on each medium and non-motile rods and cocci were present in every instance. It will be seen from Table 2 that Proske Sayers blood agar seems to be more suitable for the growth of spirochetes than any of the other media. This tends to confirm previous studies comparing Heart infusion base and the Proske Sayers base for the growth of spirochetes. All the media seemed adequate to support vibrios in mixed growth though the HIAFKE appeared to be more successful in the wells than on a flat surface. There was a tendency for filaments and filamentous fusiforms to be more prominent in the heart infusion and beef extract blood agars, than in any of the other media.

c. Since vibrios, spirochetes, and probably other organisms quite evidently grow better in the presence of the total flora than when isolated by streaking or other means, it may be that certain growth factors are elaborated by the mixed exudate. This hypothesis has been tested by one experiment and others are planned.

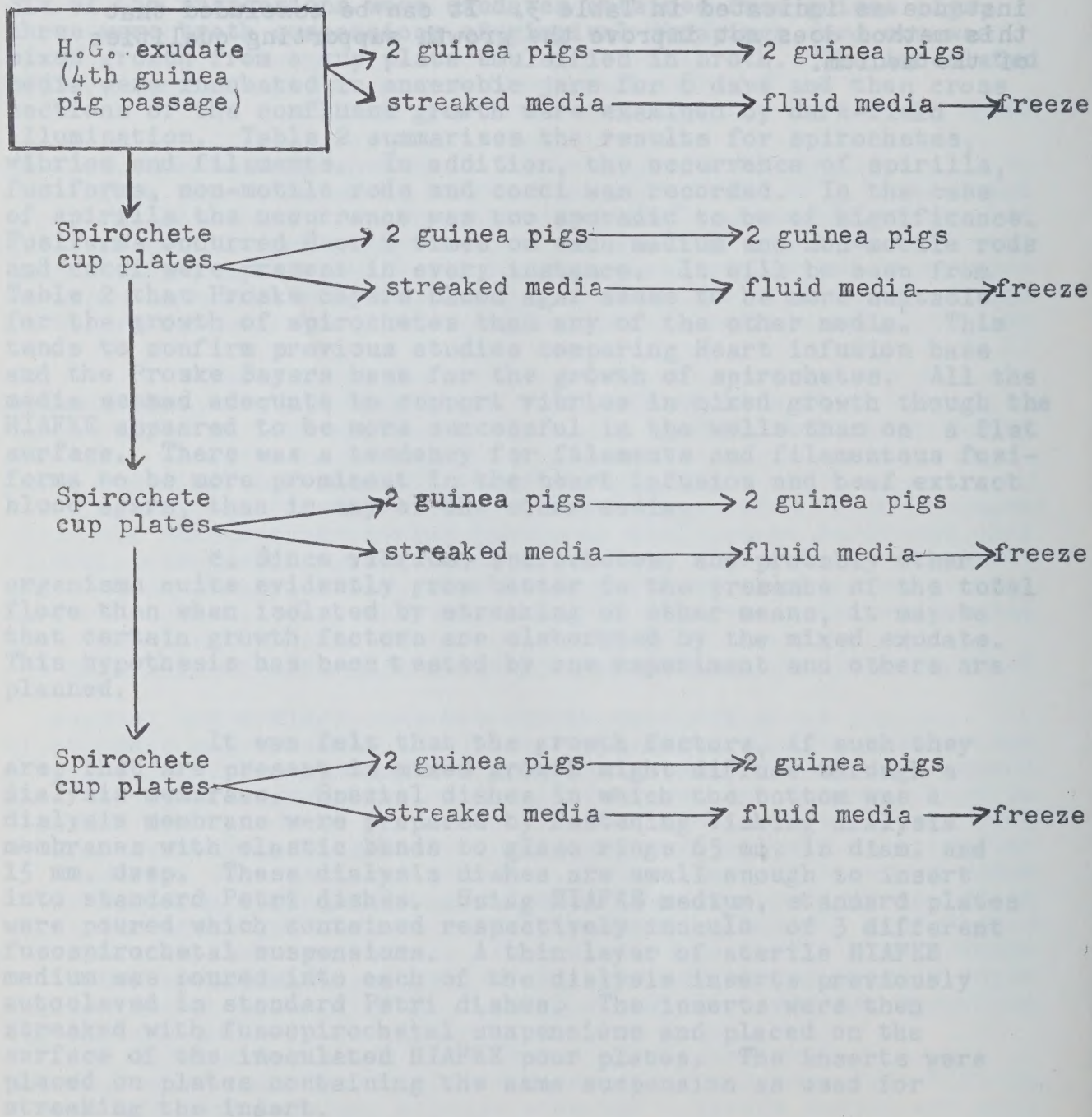
It was felt that the growth factors, if such they are, that are present in mixed growth might diffuse through a dialysis membrane. Special dishes in which the bottom was a dialysis membrane were prepared by fastening Visking dialysis membranes with elastic bands to glass rings 65 mm. in diam. and 15 mm. deep. These dialysis dishes are small enough to insert into standard Petri dishes. Using HIAFKE medium, standard plates were poured which contained respectively inocula of 3 different fusospirochetal suspensions. A thin layer of sterile HIAFKE medium was poured into each of the dialysis inserts previously autoclaved in standard Petri dishes. The inserts were then streaked with fusospirochetal suspensions and placed on the surface of the inoculated HIAFKE pour plates. The inserts were placed on plates containing the same suspension as used for streaking the insert.





Figure 1 -

SCHEME OF ISOLATION OF PURE CULTURES FROM H.G.





# DEVELOPMENT OF GUINEA PIG EXUDATE.

TABLE - I.

SOURCE 1<sup>ST</sup> PASSAGE 2<sup>ND</sup> PASSAGE 3<sup>RD</sup> PASSAGE 4<sup>TH</sup> PASSAGE 5<sup>TH</sup> PASSAGE

|                                                              |                 |                                                |                                       |                             |                |                |
|--------------------------------------------------------------|-----------------|------------------------------------------------|---------------------------------------|-----------------------------|----------------|----------------|
| D.H.<br>DILANTIN<br>HYPERPLASIN<br>D.F. - TYPICAL,<br>LIGHT. | LESION *        | +                                              | +                                     | ±                           | ±              |                |
|                                                              | DARK FIELD      | COCCI PREDOMINATE<br>ALL FORMS SEEN            | TYPICAL                               | PREDOMINATELY<br>COCCI      |                |                |
|                                                              | AEROBIC CULTURE | GREENING STREP.<br>LARGE WHITE COLONY          | GREENING STREP.<br>LARGE WHITE COLONY |                             |                |                |
| C.G.<br>CHRONIC<br>GINGIVITIS<br>D.F. - TYPICAL              | LESION *        | ±                                              | +                                     | +                           | +              | +              |
|                                                              | DARK FIELD      | TYPICAL                                        | TYPICAL                               | TYPICAL                     | TYPICAL        | TYPICAL        |
|                                                              | AEROBIC CULTURE | GREENING STREP                                 | —                                     | GREENING STREP              | GREENING STREP | GREENING STREP |
| H.G.<br>HYPERTROPHIC<br>GINGIVITIS<br>D.F. - TYPICAL         | LESION *        | +                                              | +                                     | +++                         | ++             |                |
|                                                              | DARK FIELD      | TYPICAL                                        | TYPICAL                               | TYPICAL                     | TYPICAL        |                |
|                                                              | AEROBIC CULTURE | NON HEM. STREP.<br>LARGE GREY<br>MUCOID COLONY | NON HEM. STREP                        | NONHEM. STREP.              | NONHEM. STREP. |                |
| C.P.<br>CHRONIC<br>PERIODONTITIS<br>D.F. - TYPICAL           | LESION *        | +++                                            | ++                                    | +                           |                |                |
|                                                              | DARK FIELD      | TYPICAL                                        | TYPICAL                               | TYPICAL                     |                |                |
|                                                              | AEROBIC CULTURE | GREENING STREP<br>NON HEM. STREP.              | GREENING STREP                        | LARGE GREY<br>MUCOID COLONY |                |                |
| P.E.<br>CHRONIC<br>PERIODONTITIS<br>D.F. - TYPICAL           | LESION *        | +                                              | ATYPICAL                              | +++                         |                |                |
|                                                              | DARK FIELD      | COCCI PREDOMINATE<br>ALL FORMS SEEN            |                                       |                             |                |                |
|                                                              | AEROBIC CULTURE |                                                |                                       |                             |                |                |

\* ± SMALL LOCAL LESION.

+ MODERATE LOCAL LESION.

++ LARGE LOCAL LESION.

+++ LOCAL LESION WITH NONFATAL EXTENSION IN 7 DAYS.

++++ LOCAL LESION WITH FATAL EXTENSION IN 7 DAYS OR LESS.

Table 2

Growth of spirochetes, vibrios and  
filaments in 10 mixed suspensions  
on various media.

Occurrence in 10 suspensions

Medium                      Spirochetes      Vibrios      Filaments

|                                |    |    |   |
|--------------------------------|----|----|---|
| HIAFKE cup plates              | 7  | 10 | 5 |
| HIAFKE plates                  | 6  | 7  | 5 |
| HIAFKE (filtered) plates       | 7  | 7  | 5 |
| *<br>Proske Sayers blood agar  | 10 | 10 | 5 |
| *<br>Heart infusion blood agar | 7  | 9  | 7 |
| *<br>Beef extract blood agar   | 7  | 10 | 7 |

\*10% sheep blood



Table 3 -

DIALYSIS EXPERIMENT

| Inoculum | Growth of vibrios on |                  |
|----------|----------------------|------------------|
|          | Cup plate controls   | Dialysis Inserts |
| D.M.     | +                    | 0                |
| C.P.     | +                    | 0                |
| H.G.     | +                    | 0                |

One half of the upper and lower jaw were used for autoradiographic studies. The other halves were washed, dried, crushed and sieved through 100 mesh screen and prepared according to the technique of Hodge and Smith (1938), 15:133 (1938).

Three different fractions were isolated from the powdered teeth according to specific gravities. The most active precipitates in a mixture of Sp. Gr. 2.2, 2.4 and 2.6 were the mixture's Sp. Gr. is 2.0. The intermediate fraction was active when the mixture's Sp. Gr. is 2.4 probably because of the growing enamel. The fraction containing dentin is the most active.

Intake of Vibrio  
Riverside





# APPENDIX "F"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Subject: Studies of calcium metabolism in tooth with the aid of  $Ca^{45}$  October 1950

Subject: Studies of calcium metabolism in tooth with the aid of  $Ca^{45}$

Grantee: Dr. A. D'Iorio

Project

No.: DR 34

Amount of Grant: \$2,840

### Experimental Procedure SUMMARY OF WORK

#### Animals

Newly weaned albino rats (40-50 grs) from different litters were given through stomach tube, 1 to 8 ml respectively of 0.25 M Ca lactate solution. Doses of 1 ml containing 2 microcuries  $Ca^{45}$  were administered once or twice daily and the animals killed 20 hrs after the last injection.

Animal bodies (except the jaws) were ignited at 800° C. and the ashes counted for radioactivity. Excrements both liquid and solid were treated in a similar way.

One half of the upper and lower jaws are underway for autoradiographic studies. The other halves after being fixed, dried, crushed and sieved through 100 mesh screen were processed according to the technique of Hodge and Manly (J. Dent. Res. 18:133 (1939)).

Three different fractions were isolated from the powdered teeth according to specific gravities. Coronary enamel precipitates in a mixture of Sp. Gr 2.7 Dentin precipitates when mixture's Sp. Gr. is 2.0. The intermediate fraction precipitating when the mixture's Sp. Gr. is 2.4 probably contains poorly calcified growing enamel. The fraction containing dentin is the most radioactive.

Antoine D'Iorio

Signature





# APPENDIX "F"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Studies of calcium metabolism in tooth with the aid of  $Ca^{45}$

Grantee: Dr. A. D'Iorio

Project

No. DR 34

Amount of Grant: \$2,840

### INTERIM REPORT

#### Experimental Procedure.

#### Animals

Seven newly weaned albino rats, by means of a stomachal canula, were administered one milliliter aliquots of 0.25 M radiocalcium lactate. In order to study the cumulative effect in calcifying tissues as well as to maintain a certain concentration of  $Ca^{45}$  in the blood, aliquots were all of the same volume and concentration. About 20 hours after the last injection, the animals were sacrificed at such intervals that we can have representative injected by one to eight injections (omitting five). Data concerning this procedure will be found in Table I.

TABLE I

| Rat No. | initial weight (grs.) | final weight (grs.) | No. of injections | Time elapsed between last injection and death (hrs.) |
|---------|-----------------------|---------------------|-------------------|------------------------------------------------------|
| 1       | 41                    | 41                  | 1                 | 20                                                   |
| 2       | 38                    | 36                  | 2                 | 20                                                   |
| 3       | 42                    | 43                  | 3                 | 20                                                   |
| 4       | 51                    | 58                  | 4                 | 23                                                   |
| 5       | 50                    | 59                  | 6                 | 20                                                   |
| 6       | 43                    | 53                  | 7                 | 20                                                   |
| 7       | 46                    | 55                  | 8                 | 19                                                   |



Total radioactivity

Upper and lower jaws of animals were put apart for further investigation, and the rest of the body was ashed at 800°C. Ashes were dissolved in aqua regia and diluted aliquots were used for counts and calcium determination. Counts were made with the aid of a windowless Counter +, and the Ca determinative by the Sobel and Sklersky's (1) method. Results are shown in Table II.

TABLE II

|         | Body ashes      |               | fraction 2.7 |        | fraction 2.4 |        | fraction 2.0 |        |
|---------|-----------------|---------------|--------------|--------|--------------|--------|--------------|--------|
| Rat No. | Total Ca. (mgm) | Sp.act cpm/mg | % Ca         | Sp.act | % Ca         | Sp.act | % Ca         | Sp.act |
| 1       | 263.5           | 2388          | 11.7*        | 556    | 16.6*        | 531    | 16.3*        | 802    |
| 2       | 278.5           | 3200          | 16.1*        | 1711   | 11.7*        | 1800   | 18.7*        | 2318   |
| 3       | 265.0           | 5646          | 29.0         | 817    | 38.          | 662    | 24.8         | 1493   |
| 4       | 488.0           | 1670          | 27.6         | 380    | 28.          | 380    | 23.3         | 1381   |
| 5       | 411.0           | 3998          | 28.3         | 528    | 32.          | 653    | 24.3         | 3569   |
| 6       | 369.0           | 6119          | 33.3         | 893    | 29.          | 1997   | 22.6         | 5097   |
| 7       | 455.0           | 4413          | 31.4         | 583    | 38.          | 614    | 21.9         | 3933   |

+ These values are too low to be of any significance; there must have been some interferences during solubilization of the powders.

Teeth Radioactivity

Teeth were cleaned from any adhering bone, dried, crushed and sieved through 100 mesh screen. From the powder, enamel and dentin were isolated according to Hodge and Manly's (2) technique.

As no indication was found in the literature concerning the nature of dental tissues and their corresponding specific gravities in the very young rat, the identification of tissues in relation to quotes specific gravities remains to be performed.

Histological material is ready to be sectioned and routined for autoradiography.

- 
- (1) J. Biol. Chem. 122-665- 1938
  - (2) J. Dent. Res. 18:133 (1939)



"F-3"

In Table III, total  $\text{Ca}^{45}$  distribution is given in terms of percentage of injected radioactivity.

It is obvious that no conclusions may be drawn from these values. Their inconsistency may be due to several factors, namely origin from different litters, health condition and varying ages. The same procedures will in the future be followed, applied to groups instead of individuals.

TABLE III

| Distribution of Ca<br>(as per cent of adm. $\text{Ca}^{45}$ ) |              |                    |                   |
|---------------------------------------------------------------|--------------|--------------------|-------------------|
| <u>Rat No.</u>                                                | <u>teeth</u> | <u>total ashes</u> | <u>excrements</u> |
| 1                                                             | .648         | 27.5               | 34.0              |
| 2                                                             | 1.19         | 19.5               | 39.0              |
| 3                                                             | .28          | 21.8               | 48.6              |
| 4                                                             | .53          | 8.9                | 58.3              |
| 5                                                             | .52          | 17.0               | 53.9              |
| 6                                                             | .66          | 14.1               | 48.1              |
| 7                                                             | .24          | 11.0               | 59.8              |

Geo. H. W. Lucas  
Signature





APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1950

Subject: A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia.

Grantee: Dr. G.H.W. Lucas

Project  
No.

DR 2

Amount of Grant: \$1550.

SUMMARY OF WORK

The investigations by Dr. R.J.S. Tickle on this problem were continued by Dr. R. Smithurst, beginning in May of this year. It was necessary for Dr. Smithurst to become acquainted with the technique for analysing small samples of alveolar air using the Fry apparatus. Dr. Smithurst mastered the technique very quickly and was able to analyse a number of samples of alveolar air from patients under surgical anaesthesia. From the results obtained he was of the opinion that some air enters the lungs through the mouth, in spite of mouth packing. He left Toronto in June to visit England, where he intended to gather information on nitrous oxide anaesthesia in large clinics. In October he intends to resume work on the study discontinued in June.

Geo. H. W. Lucas

Signature





APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Investigation of materials for use as cements for methyl methacrylate inlays.

Grantee: Dr. A.E.R. Westman

Project No. DR 12                      Amount of Grant: \$3,000. (includes DR 25)

SUMMARY OF WORK

Work on this project has been held in abeyance pending developments on the closely-related project DR 25 on direct acrylic fillings.

Although the direct acrylic filling materials have almost completely replaced the acrylic inlay, a cement for inlays may be essential in some cases. In view of this situation this project has been kept open until the properties of acrylic fillings have been further established.

A.E.R. Westman

\_\_\_\_\_  
Signature





## APPENDIX "I"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Investigation of repair techniques for methacrylate dentures.

Grantee: Dr. A.E.R. Westman

Project  
No. DR 21

#### SUMMARY OF WORK

This is a final report on the project which was authorized up to March 31, 1950. The experimental work was completed and described before that date but a summary report has been delayed until now because it had been expected that practical tests would be carried out.

Specifically this report is concerned with a particular type of repair - the relining of lower dentures. It is submitted that two factors are responsible for the distortion of dentures on relining - excessive heat for curing and strains induced by polymerization shrinkage of the added resin.

Practical tests were proposed but could not be completed. Based on laboratory experiments only, methods are suggested for the prevention of relining distortion for periods up to 10 months and perhaps indefinitely.

In the event that this problem might receive attention at some future date, suggestions are included for other promising lines of investigation.

A.E.R. Westman

Signature





## APPENDIX "I"

### ONTARIO RESEARCH FOUNDATION

#### Department of Chemistry

#### Dental Materials Research Fellowship

Report No. 5002 - DR 21

This is a final report on the problem of relining lower dentures. When the last report was submitted attempts were being made to arrange for practical tests of the techniques recommended for relining. However, no tests were carried out because of a lack of relined cases. The material presented in this report, then, is based entirely on laboratory experiments.

#### Denture Repairs and Relines

A report on the prevention of distortion in dentures undergoing repair was submitted at an earlier date. Two methods, based on infra-red heat and ultra-violet radiations to effect polymerization, were described and recommended for cases where the area of the denture to be repaired was small. Such cases would be fractures and the replacement of teeth.

The relining of lower dentures can not be handled satisfactorily by either of the above methods because where a relatively large bulk of new resin is added to an existing denture, stresses caused by polymerization shrinkage are great enough to produce serious dimensional changes. High temperatures should be avoided in making repairs because the release of internal stresses in dentures is an additional factor in producing distortion.

Reline distortion can be easily demonstrated and tested for by adding a thin layer of new denture base material to a flat strip of the resin, the dimensions of which are qualitatively recorded by a stone index. Figure 1(a) shows a strip and its index. It will be noted that holes have been countersunk near the ends of the test strip. Actually two holes at one end and one at the other, is the most suitable arrangement for assuring an easy and stable positioning of the index. Figure 1(b) shows, in somewhat exaggerated form, what can be observed when the strip is "relined" with regular monomer-polymer mix. Failure of the strip to fit its index is evidence that relined distortion has occurred. In practice the dimensions of the strips were approximately  $1/8 \times 1 \times 3$  inches and a relined layer about one-third of the thickness was added.

When a formulation for a relined material seemed promising an experimental denture was "relined" on a stone cast made from a master model, conditioned in water at body temperature and examined periodically for dimensional changes by fitting it on the master model.



Two methods have been suggested for preventing reline distortion. The objective of each method is to produce a relatively flexible reline resin which will not warp the original denture. Furthermore, it was considered highly desirable, if not essential, to adhere as closely as possible to the well-established and extremely simple method of using a monomer-polymer mixture as the reline material in the same way that such a mixture is used in the original processing of the denture.

### The Use of Flexible Resins

Several alkyl methacrylates such as ethyl, butyl, octyl and lauryl methacrylates were investigated. Of these, only ethyl methacrylate could be considered as a potential monomer since it alone is a good solvent for the methyl methacrylate polymer ordinarily used for dentures. It does not, however, produce with methyl methacrylate a reline material with enough flexibility. The polymers of the higher alkyl methacrylates are not ordinarily available commercially. Octyl and lauryl polymethacrylates are viscous, tacky resins and because of this are difficult to handle and cannot be used to replace methyl methacrylate polymer. These polymers were prepared from their monomers and used as 10-20% solutions in methyl methacrylate monomer as the liquid component in the monomer-polymer reline mixture. Time did not permit the preparation of butyl polymethacrylate but since the completion of the experimental work it has been learned that this polymer is available commercially as a practical grade in a form suitable, at least, for laboratory experiments in relining. Complete substitution of methyl polymethacrylate of butyl polymethacrylate would be a promising pursuit.

It was found that lauryl methacrylate produced a tacky reline and for this reason was not suitable. A 10% solution of octyl polymethacrylate in methyl methacrylate monomer, stabilized with hydroquinone, appeared to be a satisfactory liquid component for use in a reline material. This solution also contains 3% trihexylamine as a polymerization accelerator. For relines, about 0.2% benzoyl peroxide is dissolved in the above solution immediately before use and methyl methacrylate denture base polymer is added in the usual manner. After packing the denture flask, curing is carried out at 135°F for 3 1/2 hours. Test strips and experimental dentures relined in this way have remained dimensionally stable for from 8 to 10 months.

### The Use of an Inhibitor

The second method for relining dentures is also intended to produce a flexible resin. In this case a polymerization inhibitor is added to the monomer. The inhibitor would tend to produce a low-molecular-weight polymer with some degree of flexibility.



Hydroquinone, a common antioxidant, was found to be effective in preventing reline distortion only for a period of from ten days to two weeks after relining. After this time, relined strips began to warp and became progressively worse. A yellow to orange discoloration in the reline material indicated the oxidation of the hydroquinone.

A more stable compound, 2,5-ditertiary butyl hydroquinone was tried and found suitable for a considerably longer period of time. A reline monomer was formulated consisting of hydroquinone-free methyl methacrylate containing 0.05% 2,5-ditertiary butyl hydroquinone and 3% trihexylamine. Just before adding dental grade methyl methacrylate polymer to make the mixture, 0.2% benzoyl peroxide catalyst was added to the monomer and dissolved. Curing was carried out at 135° (as before, to avoid thermal distortion of the denture) for a period of 3 1/2 hours. Dentures and strips relined with this material for 3 1/2 hours appeared to be dimensionally stable for about 8 to 10 months. After this time slight distortion was noticed. If, however, the curing is prolonged for 16 hours or overnight, as is frequently done in dental laboratories, the cases are stable for longer than 8 to 10 months. Evidently the shorter curing time does not achieve complete polymerization and the formation of a high-molecular-weight polymer, with consequent distortion, can occur after a considerable time presumably when the inhibitor is oxidized. This explanation is an assumption only and no attempt has been made to prove it experimentally.

Although even the slightest distortion is undesirable, it is submitted that mouth tissues and possibly even occlusion of teeth would adapt themselves to the slowly-occurring dimensional changes which have been observed.

#### A Practical Reline Method

Summarizing what has been said above, our laboratory experiments indicate that the most suitable method for relining lower dentures can be carried out as follows: Using hydroquinone-free methyl methacrylate monomer containing 3% trihexylamine and 0.05% 2,5-ditertiary butyl hydroquinone, add immediately before mixing approximately 0.2% benzoyl peroxide and then regular denture base polymer powder. When the mixture attains a working consistency, it is packed in a dental flask in the usual manner and then cured over night or for 16 hours at 135°F. While this 16-hour method may seem unusually long, it would appear at the same time to be a practical working method in view of the fact that overnight cures for repairs are commonly employed in dental laboratories.

If, for any reason, relines need be completed in a short time, then it is suggested that a 10% solution of octyl polymethacrylate in methyl methacrylate, also containing 3% trihexylamine, be used in place of the monomer described above and that curing be carried out for 3 1/2 hours at 135°F.



In the light of recent developments in the field of dental plastics, particularly filling materials, it is quite possible that known accelerators other than trihexylamine would be more efficient and, in the manner of the so-called self-curing resins, would eliminate the necessity of heat curing. For relines, however, a flexible material would still be a requisite.

### Summary

This report is concerned with distortion of lower dentures during relining and the cause of the distortion is attributed to polymerization shrinkage of the added resin.

Methods, based on laboratory experiments only, are suggested for the prevention of reline distortion for periods of 8-10 months and perhaps indefinitely by using materials which produce flexible relines.

Other promising lines of investigation are indicated including a possible reduction in curing time and the elimination of heat curing.

Douglas R. Christie

19 Sept. 1950.



APPENDIX "A"

REPORT TO THE JOINT COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Chemical mechanisms in dental caries

Directed by: Dr. J. W. H. ...

Project No. 101 ...

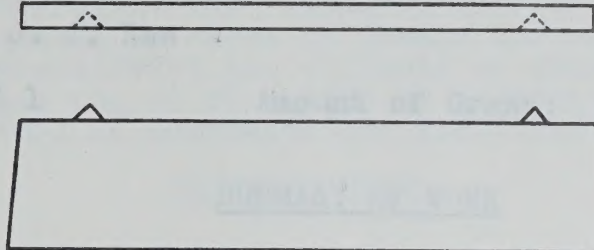
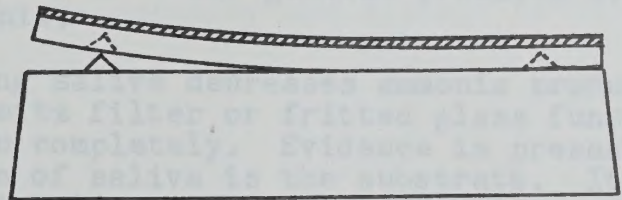


FIGURE 1(a)

(a) Ammonia Production in Saliva. (b) A Substance in Tooth Enamel prepared by the Manley and Hodge technique that inhibits the growth of lactobacillus acidophilus.

(a) Ammonia Production in Saliva. Much of the earlier work on this problem which had been reported on last February was rechecked and the work extended by Miss Hallentyne\*. There is considerable ammonia produced by saliva on incubation for 24 hours at 37°. The amount produced does not depend upon the method of collection of the saliva (stimulated or unstimulated) or (in the few cases studied) upon the l.b.c. counts of saliva. The ammonia production is inhibited by NaF (0.25%) and glucose (2.5%). Heating to 50° destroys the ammonia producing mechanism. The addition of urea to saliva produces a marked increase in ammonia production and the addition of urea and glucose produces even still larger amounts of ammonia.



Centrifuging saliva and treating the supernatant with a filter through a 0.2 µm filter or fritted glass funnel inhibits ammonia production completely. Evidence is presented that the protein fraction of saliva is the substrate. It is proposed to submit for publication this preliminary phase of the work on ammonia production.

FIGURE 1(b)

\* Through the courtesy of Consumers Research Laboratories, Toronto their excellent laboratory facilities and a competent biochemist, Miss Hallentyne, were made available for the summer months to carry on this work under my personal supervision.





## APPENDIX "J"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J. J. Rae

Project No. DR 1 Amount of Grant: \$950.

### SUMMARY OF WORK

During the last six months the work on DR 1:- Chemical Mechanisms in Dental Caries, has dealt with two phases of the work:-

(a) Ammonia Production in Saliva and (b) A Substance in Tooth Enamel prepared by the Manley and Hodge technique that inhibits the growth of lactobacillus acidophilus.

(a) Ammonia Production in Saliva. Much of the earlier work on this problem which had been reported on last February was re-checked and the work extended by Miss Ballantyne\*. There is considerable ammonia produced by saliva on incubation for 24 hours at 37°. The amount produced does not depend upon the method of collection of the saliva (stimulated or unstimulated) or (in the few cases studied) upon the l.b.a. counts of salivas. The ammonia production is inhibited by NaF (0.25%) and glucose (2.5%). Heating to 50° destroys the ammonia producing mechanism. The addition of urea to saliva produces a marked increase in ammonia production and the addition of urea and glucose produces even still larger amounts of ammonia.

Centrifuging saliva decreases ammonia production and filtering through a Seitz filter or fritted glass funnel inhibits ammonia production completely. Evidence is presented that the protein fraction of saliva is the substrate. It is proposed to submit for publication this preliminary phase of the work on ammonia production.

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\* Through the courtesy of Consumers Research Laboratories, Toronto their excellent laboratory facilities and a competent biochemist, Miss Ballantyne, were made available for the summer months to carry on this work under my personal supervision.

(b) A Growth Inhibitor for l.b.a. in Tooth Enamel. Tooth enamel prepared by the Manley and Hodge technique (bromoform to separate enamel and dentine and subsequent washing with acetone) inhibits the growth of l.b.a. The inhibition is not due to bromoform or acetone as such. The inhibitory substance can be extracted from the enamel by means of alcohol and ether. Attempts have been made to identify and fractionate the material but without notable success to date. It is not removed from the enamel by drying at 160° (B.P. of bromoform is 150°).

SUMMARY OF WORK

During the last six months the work on DR 1:- Chemical Mechanisms in Dental Caries, has dealt with two phases of the work:-

(a) Ammonia Production in Saliva and (b) A Substance in Tooth Enamel prepared by Manley and Hodge technique that inhibits the growth of l.b.a. acidophilus.

James J. Rae  
Signature

(a) Ammonia Production in Saliva. Much of the earlier work on this problem which had been reported on last February was re-checked and the work extended by Miss Ballantyne\*. There is considerable ammonia produced by saliva on incubation for 24 hours at 37°. The amount produced does not depend upon the method of collection of the saliva (stimulated or unstimulated) or (in the few cases studied) upon the l.b.a. counts of salivae. The ammonia production is inhibited by NaF (0.25%) and glucose (2.5%). Heating to 50° destroys the ammonia producing mechanism. The addition of urea to saliva produces a marked increase in ammonia production and the addition of urea and glucose produces even still larger amounts of ammonia.

Centrifuging saliva decreases ammonia production and filtering through a Geltex filter or fritted glass funnel inhibits ammonia production completely. Evidence is presented that the protein fraction of saliva is the substrate. It is proposed to submit for publication this preliminary phase of the work on ammonia production.

\* Through the courtesy of Consumers Research Laboratories, Toronto their excellent laboratory facilities and a competent biochemist, Miss Ballantyne, were made available for the summer months to carry on this work under my personal supervision.



APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Chemical Mechanisms in Dental Caries

Grantee: Dr. J. J. Rae

Project No. DR 1 Amount of Grant: \$950.

PROPOSED WORK

(a) It is intended to continue the work on ammonia production and extend it to a wider number of salivas in an attempt to find answers to these questions:-

(1) Is there a relationship between ammonia production and l.b.a. counts ?

(2) Is there a relationship between urease content of saliva and l.b.a. counts ?

(3) Is the stimulating effect of glucose on ammonia production from urea and saliva observable in all salivas ?

(4) What is the source of ammonia when salivas are incubated ?

(b) It is intended to continue the work in the inhibitory substance in tooth enamel for l.b.a. growth in an attempt to prepare it in larger quantity and attempt to identify it.

James J. Rae

Signature





## APPENDIX "J"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Chemical mechanisms in dental caries.

Grantee: J. J. Rae

Assistants: C.T. Clegg and Miss M. Ballantyne

Project: DR 1

Period covered: Feb. 1, 1950 to Sept. 15, 1950.

#### INTERIM REPORT (No.10)

The work covered in this report is divided into two sections.

(a) Ammonia Production in Saliva.

(b) An Inhibitory Substance for the Growth of l.b.a. in Tooth Enamel.

It is intended to submit a report on the first part of this work for publication in the Journal of Dental Research. Three authors appear on this paper since a part of the work was done during the summer months by Miss Ballantyne at the Consumers Research Laboratories under my personal supervision.

#### SECTION (a)

##### AMMONIA PRODUCTION IN SALIVA

The nitrogen containing constituents of saliva, particularly ammonia and urea, have been the subject of a series of papers in recent years. In 1934, Grove and Grove (1) reported a difference in the ammonia content of salivas from caries-immune and caries-susceptible persons. Several groups of workers have tried to substantiate this claim unsuccessfully (2,3,4,5). In 1943, Stephan (6) reported that the influence of carbohydrate in lowering the pH of dental plaques could be overcome by the application of 40-50% urea.

Kesel (7) has reported that filtrates from 8-day broth cultures of salivas from caries-free individuals had an inhibiting effect on the growth of lactobacillus acidophilus, whereas analogous filtrates from caries-active individuals had no such effect. He observed also that the mixture containing the filtrate from caries-immune saliva did not change in pH over 48 hours, while that from caries-active saliva permitted a drop to pH 4.2 in 48 hours. All filtrates were examined for their ammonia content and it was found that non-inhibiting filtrates contained little or no



ammonia and inhibitory filtrates contained at least 0.5 mg. ammonia nitrogen per milliliter. Cary (5) published an extensive report dealing with the presence and production of ammonia in saliva, using Conway cells to measure the ammonia. He concluded that the salivary glands have neither an excretory nor secretory function in the formation of ammonia and that the saliva owes its ammonia to the presence of bacteria. He also examined the nitrogenous material of saliva and found that most of this material is used up in 24 hours, although what he measured was the ammonia evolved, which reached a maximum in 24 hours, and not the nitrogen containing material remaining.

In order to ascertain whether there is a significant relationship between the production of ammonia in saliva and caries activity it would be necessary to examine a large number of salivas collected under uniform conditions. Such facilities were not available when this work was undertaken, therefore it was decided to investigate some of the characteristics of the mechanism of ammonia production in saliva. There seem to be two antithetic processes in saliva. (1) An acid-producing mechanism which apparently acts on carbohydrate to produce lactic acid, and which is probably due to the presence of lactobacillus acidophilus, (2) An ammonia-producing mechanism, which may also be bacterial. It is this latter mechanism that we are interested in at the present time.

### EXPERIMENTAL

The ammonia content of saliva was measured by the Permutit method of Folin and Bell (8), and the colours developed were measured in a Lumetron colorimeter. Although only a small number of salivas were studied, they all produced ammonia on standing and the amount produced did not seem to show any correlation with the l.b.a. counts of the salivas as shown in Table I.

Table I

| L.b.a. count | mg% $\text{NH}_3\text{.N}$ after 24 hours at room temperature |      |      |      |
|--------------|---------------------------------------------------------------|------|------|------|
|              |                                                               |      |      |      |
| High         | 32.8                                                          | 29.5 | 28.3 | 20.0 |
| Low          | 37.3                                                          | 45.3 | 33.4 | 31.4 |

It is intended to extend this work to a much larger group of individuals in the near future.

Cary (5) found higher ammonia values in stimulated saliva than in unstimulated after incubation for 1 hour. We repeated this work, measuring the ammonia produced in 4 and 24 hours and



found no difference (Table II). Hence, all subsequent salivas used were paraffin stimulated. We do agree with Cary however in that the maximum amount of ammonia is produced in 24 - 48 hours. The salivas were incubated at 37°C and the ammonia content measured. The relation between ammonia production and time is shown in Table II.

Table II

| Saliva       | 0 hr. | 4 hrs. | 24 hrs. | 48 hrs.                  |
|--------------|-------|--------|---------|--------------------------|
| Stimulated   | 2.4   | 9.5    | 26.7    | 29.1 mg% NH <sub>3</sub> |
| Unstimulated | 3.6   | 9.5    | 31.5    | 31.5 mg% NH <sub>3</sub> |

In a series of buffered tubes at various pH's from 7.0 to 9.5, it was found that the maximum amount of ammonia was produced at pH 8.5. When a borate buffer was used it was found to have an inhibitory effect on ammonia production.

The effect on ammonia production of the addition of sodium fluoride in concentrations varying from 1 - 10,000 p.p.m. was observed and it was found that partial inhibition was evident at 1,200 p.p.m. and that 2,500 p.p.m. produced complete inhibition. The addition of thymol to the saliva also inhibited the production of ammonia.

Temperature inhibited the production of ammonia. The inhibition at 6°C and 16°C was reversible and it was found that when the salivas were incubated at 37°C following storage for 24 hours at these low temperatures, the salivas produced as much ammonia as the control sample. However, incubation at 50°C and 70°C completely destroyed the ammonia producing mechanism.

Glucose was found to be an inhibitor of ammonia production in concentrations of 2.5%. This effect was probably due to the fact that when salivas were incubated with glucose the pH was lowered to 4.2 and at this pH the production of ammonia is decreased.

The addition of 3% urea to these salivas caused an increase in the amount of ammonia produced showing the presence of urease in saliva. However, it was interesting to note that when glucose was added to the saliva-urea mixture the amount of ammonia was at least doubled. The results are shown in Table III.

Table III

| Tube No | Saliva ml | 20% Glucose ml | 3% Urea ml | Water ml | mg% NH <sub>3</sub> saliva No.1 | in 24 hours Saliva No.2 |
|---------|-----------|----------------|------------|----------|---------------------------------|-------------------------|
| 1       | 3         | 0              | 0          | 5        | 32                              | 18                      |
| 2       | 3         | 1              | 0          | 4        | 16                              | 14                      |
| 3       | 3         | 0              | 4          | 1        | 135                             | 176                     |
| 4       | 3         | 1              | 4          | 0        | 267                             | 483                     |



In an attempt to determine whether the urease present in saliva was stimulated by the glucose, pure urease was extracted from Jack Bean Meal and its action studied on a 3% urea solution with and without added glucose. The glucose had no effect on the action of the urease. There was no significant difference in the amounts of ammonia produced by the two solutions.

Centrifuging saliva caused a reduction in the amount of ammonia produced, whereas filtering through a Seitz filter or a fritted glass funnel completely removed the ability to produce ammonia. This latter observation suggests that bacteria are responsible for ammonia production. This conclusion gains further support from the fact that the Permutit filtrates from the ammonia determination do not produce ammonia on standing even though they still contain a major portion of the total N in the saliva. Centrifuging on the other hand merely reduced the amount of ammonia produced and it was found that when total nitrogen determinations were made using a Micro-Kjeldahl procedure, the reduction in  $\text{NH}_3\text{-N}$  produced was equivalent to the nitrogen removed by centrifuging. Deakins (9) reported nearly 40% of the protein nitrogen was removed by centrifuging. This would indicate that centrifuging removes the substrate upon which the ammonia producing mechanism acts. The determination of the protein content of saliva when collected and after incubation for 24 hours at  $37^\circ$  yielded the results shown in Table IV. Trichloroacetic acid and sodium tungstate were found to be unsatisfactory for the precipitation of the protein in saliva. Uranyl acetate however gave satisfactory results.

Table IV

| Saliva                                       | Total N<br>mg % | N.P.N.<br>mg% | Protein N<br>mg%<br>(by difference) | $\text{NH}_3\text{-N}$<br>mg% |
|----------------------------------------------|-----------------|---------------|-------------------------------------|-------------------------------|
| As collected                                 | 39.2            | 8.6           | 30.6                                | 1.9                           |
| After incubation<br>(24 hrs. at $37^\circ$ ) | 39.2            | 23.4          | 15.8                                | 24.5                          |

These results would indicate that protein and probably amino acids are the substrate used in the production of ammonia.



SUMMARY

There is an ammonia producing mechanism in saliva which produces on standing, or on incubation for 24 hours at 37°C, 5-10 times the amount of ammonia originally present. Sodium fluoride (0.25%) inhibits ammonia production as does the presence of 2.5% glucose. Heating to 50°C destroys the ammonia producing mechanism. When urea is added to saliva there is an increase in ammonia production showing the presence of an active urease in the saliva. Glucose and urea added in conjunction stimulate the production of ammonia above that observed when urea alone is added, but we were unable to show that urease was stimulated by the presence of glucose.

Centrifuging the saliva decreases the ability of the saliva to produce ammonia by removing part of the substrate, whereas filtering through a Seitz or fritted glass funnel inhibits the production of ammonia completely, probably by removing the ammonia producing mechanism.

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SECTION (b)

A SUBSTANCE FROM TOOTH ENAMEL WHICH INHIBITS THE GROWTH OF LACTO-BACILLUS ACIDOPHILUS

In describing a method for determining the number of micro-organisms in rat teeth Wakeman, Smith et al<sup>(1)</sup> found that ground teeth gave a count ten times greater than when whole teeth were used. They concluded that the increase observed might be due to the micro-organisms being contained in the pits and fissures of the teeth. However it might also be concluded that ground tooth substance is a good culture media addendum for such micro-organisms. To test this assumption the following experiment was performed.

1 ml. of saliva mixed with 4 ml. of broth culture<sup>(2)</sup>, was shaken at 20° for 20 hours and 0.1 ml. of the mixture was then plated on solid tomato agar medium<sup>(3)</sup>, and incubated for 4 days at 37°. The lactobacillus count was then determined. Under the same conditions the addition of 60 mesh ground tooth enamel, prepared according to the Manley and Hodge Method<sup>(4)</sup> inhibited almost completely the growth of lactobacillus. This inhibition was not due to the shaking with beef broth for 20 hours since analogous counts were obtained from the same saliva with and without such treatment. The results of this experiment are shown in Table I.

Table I

Effect of shaking saliva and broth culture with tooth enamel on lactobacillus growth

| Saliva No. | No. Saliva | Lactobacillus in 1 cc. of Saliva<br>Saliva + 50 mg. Tooth Enamel |
|------------|------------|------------------------------------------------------------------|
| 1          | 120.000    | 0                                                                |
| 2          | 40.000     | 600                                                              |
| 3          | 15.000     | 0                                                                |
| 4          | 180.000    | 0                                                                |
| 5          | 2.000      | 0                                                                |



Our conclusion that tooth enamel would probably stimulate the growth of lactobacillus seems to be false insofar as this lot of tooth enamel is concerned. But there remains this surprising inhibiting effect of tooth enamel on the growth of lactobacillus.

Two pure cultures of lactobacillus were obtained, one of colonies on tomato agar solid media from saliva which were suspended in broth culture at 37° for 48 hours, the other from milk culture obtained from Bordens Ltd., and subcultured twice in broth culture and solid tomato agar.

The inhibitory effect may be due to the buffering effect of the tooth enamel or to the calcium or phosphate ions present. To test this assumption a comparison was made between  $\text{Ca}_3(\text{PO}_4)_2$  and the same amount of ground tooth enamel.

Test tubes containing 0.1 ml. of these cultures and 4 ml. of broth medium were prepared. To one pair of tubes 50 mg.  $\text{Ca}_3(\text{PO}_4)_2$  was added, to another pair 50 mg. of tooth enamel (Manley and Hodge), and to still another pair one whole tooth, which had been stored in 1% formaldehyde solution were added. The tubes were incubated at 37° for 24 hours, then 0.1 ml. was transferred to solid tomato agar medium and incubated for 4 days at 37°. The colonies were counted and the lactobacillus count was determined. The results are shown in Table II.

Table II

The effect of  $\text{Ca}_3(\text{PO}_4)_2$  and tooth enamel on the growth of lactobacillus

|                              | No. Lactobacillus per cc.   |                           |
|------------------------------|-----------------------------|---------------------------|
|                              | Pure Culture<br>From Saliva | Pure Culture<br>From Milk |
| $\text{Ca}_3(\text{PO}_4)_2$ | over 1 million              | over 1 million            |
| Tooth Enamel                 | 0                           | 0                         |
| Whole Tooth                  | 0                           | 36.000                    |
| Control                      | over 1 million              | over 1 million            |



Using the technique described above for testing the inhibitory effect it was found that an ethanol extract of ground tooth enamel contained an inhibitory substance and that extraction of the enamel in Soxhlet apparatus with ethanol for 3 hours completely removed the inhibitory substance from the enamel. Ether and acetone also removed the inhibitory substance.

It was found that recently extracted teeth which had not been preserved in formaldehyde did not inhibit the growth of lactobacillus nor does dentine obtained by the Manley and Hodge method.

In this method for the preparation of pure tooth enamel, the ground tooth substance is shaken with bromoform and centrifuged. The dentine is suspended in the bromoform and is poured off. The residual tooth enamel is then washed with acetone to remove the bromoform. It would appear, then, that the inhibitory effect may be due to either the bromoform or the acetone remaining on the tooth enamel. To test these assumptions the following experiments were done.

Crude tooth enamel (ground tooth substance containing both enamel and dentine) showed no inhibition of lactobacillus growth.

Crude tooth enamel shaken with acetone had no inhibitory effect, showing that adsorbed acetone was not the inhibitory substance.

When the crude tooth enamel was shaken with bromoform, centrifuged, the bromoform and dentine decanted, the residue dried and not washed with acetone, the enamel so produced completely inhibited lactobacillus growth (zero count). When bromoform was shaken with  $\text{Ca}_3(\text{PO}_4)_2$ , the  $\text{Ca}_3(\text{PO}_4)_2$  so treated showed no inhibitory effect on lactobacillus growth, showing that it is unlikely that bromoform per se is the inhibitor. This was further substantiated by the fact that, in two experiments 0.07% bromoform and 3.6% bromoform still permitted growth of lactobacillus.

Since the inhibitory substance is not  $\text{Ca}_3(\text{PO}_4)_2$  and is soluble in organic solvents it is possible that the organic fraction of whole teeth may contain the inhibitory substance.

Whole teeth were decalcified by immersion in 5% nitric acid for 14 days. The organic residue from this treatment, when shaken with bromoform, separated and dried, showed no inhibition of growth of lactobacillus.

When bromoform-treated tooth enamel was heated at  $160^\circ$  for 30 minutes the inhibitory substance was still present and active. The boiling point of bromoform is  $150^\circ$ . The bromoform washings produced in the Manley and Hodge technique, when dried at  $160^\circ$ , contained no inhibitory substance.



Hot water does not extract the inhibitory substance but seems to inactivate it, leaving the enamel with no inhibiting effect. Subsequent drying of the enamel and shaking with bromoform produces an inhibitory substance. In the same experiment it was found that tooth enamel previously exhausted of its inhibiting substance by extraction with 95% ethanol, when shaken again with bromoform, produced more of the inhibiting substance.

Based on the above information the following method of preparation for the inhibitory substance and extraction was devised.

25 gm. of tooth enamel was shaken with 50 ml. of bromoform in a 100 ml. volumetric flask for 15 minutes. The enamel, after separating from the bromoform by filtering through No. 1 filter paper, was dried in an oven at 160° for 30 minutes. The inhibiting substance was extracted in a Soxhlet apparatus, using 150 ml. of 95% ethanol or ether, for 3 hours. The alcohol or ether was evaporated with heat leaving the inhibiting substance as a residue.

Qualitative tests for elements showed that the inhibiting substance contained no sulphur, nitrogen, phosphorus, bromine or iodine, but did contain some halogen ion, probably chloride.

An alcoholic extract of the inhibiting substance showed 1.55 mg. calcium in 323 mg. of residue. Of this residue, it was found that only 190.2 mg. dissolved in ether and that the ether-soluble fraction contained only a trace of calcium, although it still showed its inhibitory properties.

An alcoholic extract was made of a sample of tooth enamel previously exhausted of inhibiting substance with alcohol, and a comparable amount of calcium was found in this alcoholic extract. Apparently the calcium in the extract is not an essential part of the inhibitory substance and it was decided that ether would be a better extracting agent.

The inhibiting substance was found to melt at 16.4° but it decomposed on heating.

The inhibitory substance contained in an ether extract was classified according to Kamm's Qualitative solubility tests<sup>(5)</sup>. It was found that the inhibiting substance was insoluble in water, dilute hydrochloric acid, dilute sodium hydroxide, cold conc. sulphuric acid and soluble in ether. Therefore it is classified in Group VI, which Group contains aliphatic and aromatic hydrocarbons and their halide derivatives.

It is proposed to continue this very interesting work.



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APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: A study of the calcifying potential of calcium oleate on the dental pulp.

Grantee: Dr. H. A. Hunter

Project No. DR 33

Amount of Grant: \$1500

SUMMARY OF WORK

The laboratory work on this project was started this past July and to-date, six experimental and six control teeth in dogs have been used. Both buccal and occlusal pulp exposures were made and capped with a paste made of calcium oleate in oleic acid and covered by Denco temporary cement. The animal was sacrificed 15 days later and sections are in preparation. Other teeth are to be treated at different time intervals and compared with controls as well as with known calcifying agents.

H. A. Hunter

Signature





## APPENDIX "L"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth.

Grantee: Dr. J. W. Abbiss and Dr. A.G. Nutlay

Project No. DR 27 Amount of Grant: \$200.

#### SUMMARY OF WORK

In this study the enamel, dentin and cementum are treated under separate headings.

#### E N A M E L

In the delicate cells of the enamel organ autolysis takes place rapidly and much artefact is produced by most methods of decalcification. This has largely been overcome by using the method of Boedecker where ground sections are embedded in celloidin decalcification. Radiographic control to determine the end-point of decalcification has been used. By this improved technique and the use of serial sections a start has been made to our work and in the future radiological examination (Grenz Ray) and polarised light will be used as additional methods. It is hoped that the line of approach will give information as to the origin, nature and relationship of the interprismatic substance to the enamel rod.

#### D E N T I N E

The outermost layer of the dentin which in the beginning is argyrophil does not show in our specimens (fig. 1) that collagenous fibres originate from an alteration in the argyrophil fibres. The presence of epithelial cells (ameloblasts) may be the determining factor in preventing the development of collagen.

#### C E M E N T U M

Origin and function associate cementum with the tooth surrounding organs, i.e. bones and periodontal membrane. An intensive study of adult and embryonic bone structure is added to this study of the histology and cementum and periodontal membrane. The tensile force of the periodontal membrane acting upon the internal surface of the dental alveoli deletes the lamellar structure of the alveolar bone and thus it forms a tissue similar to cementum. In this study cementum is considered as a medium through which stimuli and functional connection are conducted from the environment of the tooth in an alternating course.



Legends to illustrations:

Fig. 1 Haematoxylin eosin.

This method of staining (Harris) gives a good general picture of celloidin embedded sections. In this picture an epithelial strand turns aside from the course of the enamel epithelium along the long axis of the tooth germ. This is the lamina of the permanent tooth.

Fig. 2 Bielschowsky -Maresch.  
Silver Gold.

The epithelial strand is in a continuous line, whereas the former picture showed an interrupted course. v.Korff's argyrophil fibres are clearly distinguished in this picture. Gold chloride was used in this preparation in order to avoid an overstaining of the dentin which occurs if the silver method is used alone.

A.G. Nutlay

Signature

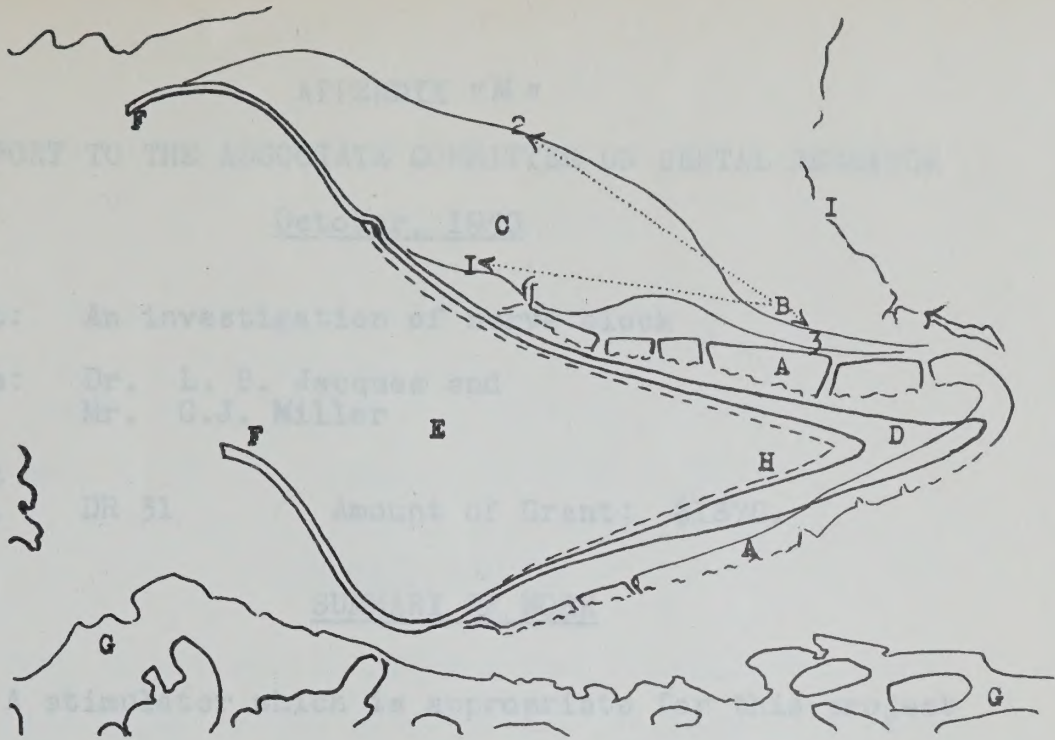


REPORT TO THE ASSOCIATE CHIEF OF DENTAL SERVICE

Subject: An investigation of the development of the tooth germ

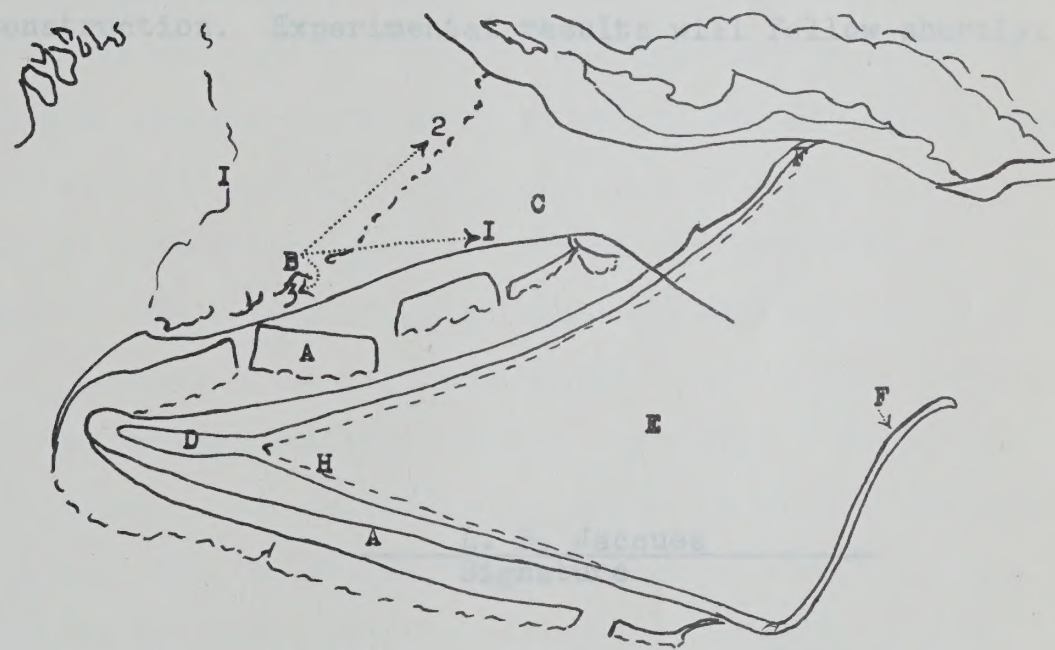
Grantee: Dr. L. B. Jacques and Mr. G. J. Miller

Project No. DR 51 Amount of Grant \$10,000



has been constructed and appropriate electrical facilities are

under construction. Experience with the use of the facilities



- |                               |                                |
|-------------------------------|--------------------------------|
| A---Dentin                    | E---Pulp                       |
| B1--Inner Enamel Epithelium   | F---Epithelial Sheath (Loop)   |
| B2--Outer Enamel Epithelium   | G---Bone                       |
| B3--Reduced Enamel Epithelium | H---Odontoblasts               |
| C---Stellate Reticulum        | I---Dental Lamina of permanent |
| D---Pre Dentin                | Tooth Germ                     |





APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: An investigation of nerve block

Grantee: Dr. L. B. Jacques and  
Mr. G.J. Miller

Project No. DR 31 Amount of Grant: \$1870.

SUMMARY OF WORK

A stimulator which is appropriate for this project has been constructed and appropriate electrical facilities are under construction. Experimental results will follow shortly.

L. B. Jacques  
Signature





APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: An investigation of nerve block

Grantee: Dr. L. B. Jacques and  
Mr. G. J. Miller

Project No. DR 31                      Amount of Grant: \$1870

INTERIM REPORT

This investigation assumes that occasional failure of Procaine block of the inferior dental nerve depends upon an alteration in the sensitivity of pain endings in the tooth which leads to the production of nerve impulses at high frequency at the block. The investigation therefore required the construction of a stimulator by which a nerve could be stimulated at various known frequencies. This stimulator must be of such design that the frequency of stimulation does not affect the intensity of stimulation. The construction of such a stimulator has been completed the week preceding this report. It has been found that our new Medical Building creates considerable static on our oscilloscope and appropriate screening facilities are under construction. Mr. Joe Hromek, an electronics technician, has been employed to assist in this research as of July 1, 1950. Experimental details must await the next report.

G. J. Miller





## APPENDIX "N"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Biological absorption of fluorine.

Grantee: M. Doreen Smith

Project No. DR 29 Amount of Grant: \$375.00

#### SUMMARY OF WORK

A paper embodying previous work reported to the Associate Committee on Dental Research was published in the Canadian Journal of Research, Vol. 28, No. 7, July 1950.

Fluorine balance experiments were carried out with a young male infant, the first two-day period at 1 1/2 months of age and the second two-day period at 4 months of age when the child was receiving 9 tbsp. Pabulum per day in addition to formula. Balance experiments were also done with three young women. In the first three-day period, a normal diet was consumed, in the second period, one cup of Pabulum per day was included in the diet and in the third 6-9 cups of tea were drunk.

In these experiments, samples of all foods ingested were obtained for analysis and pooled for each 24 hour period. Also all excreta - urine and faeces - were collected and prepared for storage prior to analysis.

Because of high blanks in the fluorine determination, analysis of these materials had to be stopped till the cause of these high blanks was discovered ( see report of project DR 13 ). However, the following results were obtained:

|        |          |         |                |           |
|--------|----------|---------|----------------|-----------|
| Infant | Period I | Formula | -670 ml/day    | 69.5 ug.  |
| Adults | Period I | Meals   | Dinner No. 1   | 21.6 ug.  |
|        |          |         | Breakfast No.1 | 131.6 ug. |
|        |          |         | Lunch No. 1    | 270.1 ug. |

The results of the completed study will be reported later.

Various materials were tested as to their value in dissolving urine ash, since the distillation of this ash is complicated by the necessity of adding a considerable amount of silver sulphate to precipitate the chlorides present. It was hoped that the ash could be dissolved, the chlorides precipitated and filtered off before distilling, but no suitable dissolving agent was found.

Using a 2% sodium hydroxide solution and drawing the air in through neoprene tubing, a rough estimation of atmospheric fluoride was made. However it is believed that a longer sampling period is necessary and perhaps a greater air flow for dependable results.

M. Doreen Smith  
Signature





## APPENDIX "N"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Biological absorption of fluorine

Grantee: M. Doreen Smith

Project: DR 29                      Amount of Grant: \$375.

A paper entitled "Fluoride Studies Related to the Human Diet" was published in the Canadian Journal of Research, Vol. 28, No. 7, July, 1950. The work reported in this paper was supported by a grant from the Associate Committee on Dental Research of the National Research Council and was contained in previous reports sent to the Committee.

Subsequent to this study of the fluoride content of food, it seemed of interest to determine the absorption and retention of this fluoride when ingested by humans, especially growing humans. This necessitated setting up balance studies.

Balance experiments were performed on a male infant. In the first two-day period, the child was a month and a half old and receiving a formula consisting of water, evaporated milk, dextro-maltose and ABC drops. In the second two-day period, the child was four months old and receiving formula plus nine table-spoons of Pabulum per day. Pabulum had been introduced gradually to the diet of this child and built up to this level. The Pabulum used in this study is quite high in fluoride having a content of approximately 12 p.p.m. The greater part of this is assumed to come from bonemeal which constitutes 2% of the Pabulum.\* Presumably the larger part of the fluoride in Pabulum is present as calcium salts and there appears to be some doubt as to the extent of absorption of fluorine in this form. This balance study was planned to give some information on this question.

Duplicates of the formulae were obtained and all urine and faeces were collected separately for the periods. This was done by means of a urine adapter and a special hammock designed for balance experiments in which the child lay during the course of the study. Carmine was used to mark off the faeces of each period.

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\* Since this work commenced the processing of Pabulum has been changed. Analyses on this new type Pabulum shows the fluoride content to be 6.1 p.p.m. Enough of the old stock was on hand in the Dept. for use in our balance experiments.





## "N-2"

Balance experiments with three young women were also carried out. In the first three day period a normal diet was consumed. In the second three day period a diet including one cup of Pablum per day was eaten. In the third three day period the diet included from 6-9 cups of tea per day. These periods were preceded by two day pre-periods in which the diet for the following period was consumed.

All food was weighed on serving, a 1/4 portion collected for each meal and pooled for each three meals. The water ingested was measured and calculated separately.

All excreta were also collected for the three day periods. Urine was voided just before the first meal of the experiment and discarded and then the urine was collected for each 24 hour period. The urine was preserved with toluene. Faeces were also collected, dried in the air oven at 70°C, pooled for each period and stored. Norite or carmine, taken at the beginning of the first meal of the period and at the first meal after the end of the experiment, was used to mark the faeces of each period.

After a small number of determinations had been made on the material from these series of experiments, the blank in fluorine determinations became high. A number of experiments were carried out to determine the cause of this high blank and these have been fully reported to the Associate Committee on Dental Research under Grant No. DR 13. It is now believed that we can proceed with the analyses.

Results obtained to date on the balance experiments are as follows:

|                                  |                             |
|----------------------------------|-----------------------------|
| <u>Infant</u> Period I - Formula | -670 ml./day = 69.5 ug.     |
| <u>Adults</u> Period I - Meals   | -Dinner No. 1 = 21.6 ug.    |
|                                  | -Breakfast No.1 = 131.6 ug. |
|                                  | -Lunch No. 1 = 270.1 ug.    |

Results of these metabolism studies will be reported to the Associate Committee when completed.

In preliminary fluorine analyses of urine, it was found that approximately 10 gm. of silver sulphate was necessary to precipitate the chlorides and reduce the acidity of the distillate to an acceptable level. However, this amount of precipitate in the distilling flask causes considerable bumping and various means of reducing this were investigated. It was thought that the ash might be dissolved, the chlorides precipitated and filtered off. The ash did not dissolve in 60% perchloric acid, concentrated hydrochloric acid, 40% sodium hydroxide solution or concentrated ammonium hydroxide unless diluted with a very considerable amount of water. Dilute Phosphoric acid seemed to dissolve the ash, but the phosphates interfere in the subsequent determination.





"N-3"

Until the difficulties arose with the fluorine determination work also proceeded on the measurement of atmospheric fluorine. In addition to the absorbing materials discussed in the previous report (March 1950) Norite and distilled water were tested as absorbing agents, but without success. Consequently, we again used a 2% sodium hydroxide solution which had previously been recommended. Outside air was drawn into the alkaline solution through neoprene tubing (chemically inert) since it was felt that in previous experiments of this type, the fluorine may have been adsorbed on the tubing used in bringing in a sample of outside atmosphere. The sampling lasted for 1 3/4 hours and it was found that the air contained approximately 0.11 p.p.m. fluorine.

It is believed that the period of sampling was not long enough to obtain dependable results and an eight hour period is planned. Through correspondence with Mr. T. Bonney of the Aluminum Company of America, it was learned that their technique for atmospheric fluorine analysis is similar to ours except that they use 0.5% sodium carbonate or distilled water as an absorbing agent and draw a greater volume of air through it over a longer period of time.

M. Doreen Smith

Signature





## APPENDIX "O"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October 1950

Subject: The study of the closure of space following premature loss of deciduous teeth.

Grantee: Norma Ford Walker

Project: DR 30

#### SUMMARY OF WORK

1. In this group of children four fifths showed loss of space after premature loss of deciduous molars. In girls, upper spaces had greater loss than the lower. The amount of loss showed no difference between sexes, and varied from 0.1 to 7.8 mm. It was also found the space lost in greater amount if more than one tooth were extracted. The non-extraction quadrants were affected by the extraction one on the opposite jaw.
2. Second molars or first and second molars had greater loss. The upper lost about as twice or one third more than the lower. Lower first deciduous molar space had the smallest loss.
3. Space lost in greater amount in the first six months after the extraction or during the eruption of permanent anteriors. Spaces in some cases reopened when permanent bicuspid were erupting.
4. More upper first permanent molars showed mesial moving and more lowers mesial tipping. This mesial moving or tipping was accompanied mostly with lingual or buccal rotation.
5. Eruption of permanent dentition, except first permanent molars and permanent central incisors, in this group of children is much earlier than that of Hellman's data. The sequence of eruption is irregular and several permanent teeth may come in at once.
6. All widths of arches, except the upper anterior width, were smaller than those of Cohen's. Continuous increase in anterior arch width on both jaws were found in two thirds or four fifths of the cases in younger group and one half in older group. Continuous increased posterior width was found in one third of the upper and one seventh of the lower. This indicates that the growth of the anterior width is not affected by the premature loss. The posterior width may grow normally but it is masked by the mesial movement of the first permanent molar.





## APPENDIX "O"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: The study of the closure of space following premature loss of deciduous teeth.

Grantee: Norma Ford Walker

Project: DR 30

#### Introduction

Willet (1934), Cohen (1941), Brauer (1941) and Schacter (1943) have studied the incidence of closure of space from premature loss of deciduous teeth and reported different results. For instance, Brauer reported that one third of the cases with early loss of first deciduous molars had malposed first bicuspids, but Cohen said that only one in eighteen showed malposition of first bicuspids. These conflicting results makes one doubt whether a space maintainer is necessary for the early loss of the first deciduous molars. MacGregor (1945) stated that if any deciduous teeth, except upper anteriors, were lost early, their spaces would close and the permanent dentition might be affected. In order to evaluate these statements, the present study was set up to find out the incidence, rate, amount and mechanism of closure of space following premature loss of deciduous teeth.

#### Findings and Data Collected

Because of the close relation between the width of the arch, the eruption of permanent dentition and the dimension of the space, the changes in all three were observed.

A total of 720 models have been measured. The number of deciduous molars extracted is 111, 45 in girls and 76 in boys. A total of 89 quadrants was involved, 32 in girls and 59 in boys. The following findings and data were obtained:

A. Changes in Space: The changes in non-extraction and extraction quadrants of mouths of boys and girls were studied. In majority of the cases in non-extraction group, the antero-posterior dimension decreased in both sexes and at different ages (in girls, 14 or 87.5% showed decrease and 2 or 12.5% increase; in boys, 23 or 74.2% decrease and 8 or 25.8% increase). The change of space in this group showed no significant difference between boys and girls. In girls, of the extraction group, 26 quadrants or 81.2% showed loss of space and only 6 or 18.8% increased. The upper ones had greater losses or gains. The girls in Class II (unilateral extraction on both jaws) and in Class IV (unilateral extraction on one jaw and bilateral on the other) had greater loss. In boys, 44 out of 52 or 84.6% had loss of space and 8 or 15.4% increase, 5 quadrants could not be measured. In this group there was no definite difference between uppers and lowers as in girls. The upper and lower arch in Class II (unilateral extractions on both jaws) and Class IV (unilateral



on one jaw and bilateral on the other) and Class V (bilateral on both jaws) showed greater decrease (more than 3 mm.). The amount of increase in space in boys were much smaller than decrease. Comparing the loss in the sexes no definite difference was found.

Comparing the various teeth, the space of lower first deciduous molar had the smallest loss in the two groups (1.7 mm. in younger children and 0.5 in olders). The loss of space in upper first or second deciduous molars was greater than that of the lowers, especially in the younger group. The spaces for second deciduous molars or first and second deciduous molars showed greater loss in both jaws. In the older group, 9 spaces had increase and 5 in the younger children as well. This will be shown in Table III.

The rate of closure of space in 59 recent extraction quadrants was observed. Out of 59 quadrants, 27 or 45.8% had greatest loss of space in 2 to 3 months after the operation. Of these cases, 16 were in 6-6.9 year group, their permanent teeth were not all erupted or not entirely in position, the other 11 were in the 7-year-and-up group and some of their permanent incisors were not fully erupted. Continuous loss of space for a half year after extraction was observed in 9 or 15.2%. Slight loss after extraction, but with increased loss during the eruption of permanent incisors was observed in 8 or 13.6%. In 13 or 22.2% the space decreased irregularly and then increased because of the eruption of the permanent bicuspid. In only one case the space increased gradually throughout the whole period of observation.

In studying the process of closure of space, it is found that the main factor involved is the movement of the first permanent molar and of the cuspid. Since the arch expands laterally, the teeth move laterally or buccally with the arch. In this study, we assume that the cuspid moves laterally when the anterior width increased, and that the cuspid tips lingually when the width decreases. Also we assume that the cuspid tips distally when the space loss is great at the time of the eruption of anteriors. The movement of the first permanent molar is determined from the measurements of the width between these teeth, and from the three measurements taken from the cuspid to the first permanent molar and from the position of the cuspid. From these observations it is found that 34 upper (18 in non-extraction and 16 in extraction quadrants) and 23 lower (10 in non-extraction and 13 in extraction quadrants) first permanent molars showed mesial movement with lingual rotation. Also 13 upper (9 in non-extraction and 4 in extraction quadrants) and 15 lower (6 in non-extraction and 9 in extraction quadrants) showed mesial movement with buccal rotation. In the extraction quadrants, 4 upper and 11 lower first permanent molars had mesial tipping with lingual rotation, and only one in the non-extraction group showed the same movement. None first permanent molars (1 upper and 8 lower) with extracted deciduous molars had mesial movement with buccal rotation. In the lower jaw, 2 had mesial tipping, 4 showed mesial moving, and 3 had lingual rotation. Distal moving and distal moving with lingual or buccal rotation was found in 3 lower molars and 6 uppers. See Table V.



B. Eruption of Permanent Dentition:

The sequence of eruption of the permanent dentition is irregular and most of the present cases do not follow Hellman's sequence curve. In some cases, several permanent teeth erupt at the same time and the sequence of eruption cannot be determined.

It is also found the natural exfoliation of the deciduous molars in this group of children occurs early so that their permanent successors also come in position early.

A comparison of the eruption of the permanent dentition in boys and girls of the present study was made with Hellman's data. It was found that all the permanent teeth, except the upper and lower first permanent molars and central incisors, were in position earlier than those of Hellman's series and this was especially true of the bicuspid and lower cuspids which erupted very much earlier (2 to 3 years). Since the observation is not completed at the moment, we cannot say whether there is a significant difference between these two groups of children.

C. Changes of the Width of Arch:

Table VIII shows the changes in width of arch. It was found that among 18 younger children, 12 of them had continuously increased upper anterior width, and 15 had continuously increased lower. In the older group about half of them showed continuous increase. In the upper posterior width, 15 of 35 showed continuous increase and the remaining 20 showed no changes or decreases. In the lower jaw, continuously increased posterior width was found in 5 of 35 cases. The increase of anterior width in majority of the cases indicates that the growth of the anterior part of the jaw is not affected by the premature loss of deciduous molars. The posterior part of the jaw may be growing normally but its growth is masked by mesial movement or drift of the first permanent molar. Therefore, no changes or decrease of the width may occur. The widths between the first deciduous molars or bicuspid and between the second deciduous molars or bicuspid showed fluctuations and decreases in half of the cases. Mostly the decrease occurs when the bicuspid are coming in position or when the bicuspid crown has started its intraosseous eruption as shown in the X-rays.



TABLE I Showing the changes of spaces in mm. in non-extraction and extraction groups in girls of different ages. Each quadrant of the mouth is used as a case unit.

| <u>Upper Arch</u> |   |       |      |      |      | <u>Lower Arch</u> |                   |  |       |      |      |      |       |
|-------------------|---|-------|------|------|------|-------------------|-------------------|--|-------|------|------|------|-------|
| No. Inc.<br>of or |   | Cases | Dec. | Max. | Min. | Aver.             | No. Inc.<br>of or |  | Cases | Dec. | Max. | Min. | Aver. |
|                   |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| Non-extract.      |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| Groups            |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| 6 - 6.9           | 9 |       | dec. | 2.4  | 0.5  | 1.4               | 1                 |  | dec.  |      |      |      | 0.8   |
|                   | 1 |       | inc. |      |      | 0.4               | -                 |  | -     | -    | -    | -    | -     |
| 7 year up         | 3 |       | dec. | 1.1  | 0.2  | 0.7               | 1                 |  | dec.  |      |      |      | 0.1   |
|                   | 1 |       | inc. |      |      | 0.6               | -                 |  | -     | -    | -    | -    | -     |
| Extraction        |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| Groups            |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| Class I           | - |       | -    | -    | -    | -                 | -                 |  | -     | -    | -    | -    | -     |
| Class II          |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| 6 - 6.9           | 1 |       | dec. |      |      | 3.2               | 1                 |  | dec.  |      |      |      | 1.1   |
| 7 year up         | 2 |       | dec. | 5.2  | 1.7  | 3.5               | 2                 |  | dec.  | 0.5  | 0.4  |      | 0.5   |
| Class III         |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| 6 - 6.9           | - |       | -    | -    | -    | -                 | 7                 |  | dec.  | 4.2  | 0.8  |      | 2.9   |
|                   | - |       | -    | -    | -    | -                 | 1                 |  | inc.  |      |      |      | 0.4   |
| 7 year up         | - |       | -    | -    | -    | -                 | -                 |  | -     | -    | -    | -    | -     |
| Class IV          |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| 6 - 6.9           | 1 |       | dec. |      |      | 3.2               | 2                 |  | dec.  | 3.4  | 0.8  |      | 2.1   |
| 7 year up         | 2 |       | dec. | 2.6  | 1.8  | 2.2               | 4                 |  | dec.  | 3.5  | 1.5  |      | 2.3   |
| Class V           |   |       |      |      |      |                   |                   |  |       |      |      |      |       |
| 6 - 6.9           | - |       | -    | -    | -    | -                 | -                 |  | -     | -    | -    | -    | -     |
| 7 year up         | 2 |       | dec. | 1.4  | 1.4  | 1.4               | 1                 |  | dec.  |      |      |      | 0.3   |
|                   | 2 |       | inc. | 4.9  | 4.1  | 4.5               | 3                 |  | inc.  | 3.8  | 0.5  |      | 1.9   |

\* Inc. - increase  
Dec. - decrease



TABLE II Showing the changes of spaces in mm. in non-extraction and extraction groups in boys of different ages. Each quadrant of the mouth is used as a case unit.

| <u>Upper Arch</u>     |      |      |                   |       |       | <u>Lower Arch</u> |      |      |       |     |
|-----------------------|------|------|-------------------|-------|-------|-------------------|------|------|-------|-----|
| No. Inc.<br>of or     |      |      | No. Inc.<br>of or |       |       |                   |      |      |       |     |
| Cases                 | Dec. | Max. | Min.              | Aver. | Cases | Dec.              | Max  | Min. | Aver. |     |
| Non-extraction Groups |      |      |                   |       |       |                   |      |      |       |     |
| 6 - 6.9               | 10   | dec. | 2.2               | 0.1   | 0.8   | 7                 | dec. | 1.3  | 0.2   | 0.6 |
|                       | 4    | inc. | 1.3               | 0.1   | 0.7   | -                 | -    | -    | -     | -   |
| 7 year up             | 4    | dec. | 1.3               | 0.1   | 0.7   | 2                 | dec. | 3.2  | 0.9   | 2.1 |
|                       | 3    | inc. | 0.5               | 0.1   | 0.3   | 1                 | inc. |      |       | 0.1 |
| Extraction Groups     |      |      |                   |       |       |                   |      |      |       |     |
| Class I               |      |      |                   |       |       |                   |      |      |       |     |
| 6 - 6.9               | -    | -    | -                 | -     | -     | 2                 | dec. | 3.1  | 2.2   | 2.7 |
| 7 year up             | 1    | dec. |                   |       | 0.6   | 1                 | dec. |      |       | 1.0 |
| Class II              |      |      |                   |       |       |                   |      |      |       |     |
| 6 - 6.9               | 3    | dec  | 3.6               | 2.1   | 3.0   | 3                 | dec. | 3.0  | 2.0   | 2.6 |
| 7 year up             | -    | -    | -                 | -     | -     | -                 | -    | -    | -     | -   |
| Class III             |      |      |                   |       |       |                   |      |      |       |     |
| 6 - 6.9               | 2    | dec. | 2.9               | 0.3   | 1.6   | 4                 | dec. | 2.2  | 0.9   | 1.8 |
|                       | -    | -    | -                 | -     | -     | 2                 | inc. | 0.3  | 0.1   | 0.2 |
| 7 year up             | -    | -    | -                 | -     | -     | 2                 | dec. | 4.3  | 2.0   | 3.2 |
| Class IV              |      |      |                   |       |       |                   |      |      |       |     |
| 6 - 6.9               | 4    | dec. | 5.9               | 1.0   | 3.2   | 5                 | dec. | 2.5  | 0.7   | 1.3 |
| 7 year up             | 2    | dec. | 4.3               | 1.6   | 3.0   | 3                 | dec. | 3.6  | 2.3   | 3.1 |
| Class V               |      |      |                   |       |       |                   |      |      |       |     |
| 6 - 6.9               | -    | -    | -                 | -     | -     | -                 | -    | -    | -     | -   |
| 7 year up             | 8    | dec. | 3.7               | 0.2   | 2.2   | 4                 | dec. | 7.8  | 1.5   | 3.6 |
|                       | 1    | inc. |                   |       | 0.7   | 5                 | inc. | 0.8  | 0.1   | 0.5 |

TABLE III Showing changes in space following extraction of specific teeth in upper and lower jaw.

| Age Group | Extracted Teeth                   | Amount of decrease in mm. |      |      |       | Amount of increase in mm. |      |      |       |
|-----------|-----------------------------------|---------------------------|------|------|-------|---------------------------|------|------|-------|
|           |                                   | No. Cases                 | Max. | Min. | Aver. | No. Cases                 | Max. | Min. | Aver. |
| 6-6.9     | Upper 1st dec.molar               | 2                         | 5.9  | 0.3  | 3.1   | -                         | -    | -    | -     |
| 7 year    | "                                 | 4                         | 2.6  | 0.2  | 1.5   | -                         | -    | -    | -     |
| 6-6.9     | Lower 1st deciduous molar         | 5                         | 3.1  | 0.8  | 1.7   | 2                         | 0.4  | 0.1  | 0.3   |
| 7 year    | "                                 | 1                         |      |      | 0.5   | 1                         |      |      | 0.2   |
| 6-6.9     | Upper 2nd deciduous molar         | 5                         | 3.6  | 2.9  | 3.2   | -                         | -    | -    | -     |
| 7 year    | "                                 | 7                         | 5.2  | 1.6  | 2.9   | 1                         |      |      | 0.7   |
| 6-6.9     | Lower 2nd deciduous molar         | 14                        | 4.1  | 0.9  | 2.2   | -                         | -    | -    | -     |
| 7 year    | "                                 | 6                         | 4.3  | 0.4  | 2.2   | 2                         | 0.5  | 0.3  | 0.4   |
| 6-6.9     | Upper 1st and 2nd deciduous molar | 4                         | 3.6  | 1.0  | 2.2   | -                         | -    | -    | -     |
| 7 year    | "                                 | 6                         | 4.1  | 1.6  | 2.0   | 2                         | 4.9  | 4.1  | 4.5   |
| 6-6.9     | Lower 1st and 2nd deciduous molar | 5                         | 4.2  | 0.7  | 2.4   | 1                         |      |      | 0.3   |
| 7 year    | "                                 | 11                        | 7.8  | 0.3  | 2.7   | 4                         | 3.8  | 0.1  | 1.2   |



TABLE V Showing the type of movement of first permanent molar during the process of closure of space. Each quadrant of a mouth is used as a case unit.

| Movement of first Permanent molar.   | <u>Non-Extraction Group</u> |      |        |      | <u>Extraction Group</u> |      |        |      |
|--------------------------------------|-----------------------------|------|--------|------|-------------------------|------|--------|------|
|                                      | Upper                       |      | Lower  |      | Upper                   |      | Lower  |      |
|                                      | Female                      | Male | Female | Male | Female                  | Male | Female | Male |
| Mesial moving with lingual rotation  | 10                          | 8    | 2      | 8    | 7                       | 9    | 1      | 12   |
| Mesial moving with buccal rotation   | 3                           | 6    | 1      | 5    | 2                       | 2    | 1      | 8    |
| Mesial tipping with lingual rotation |                             | 1    |        |      |                         | 4    | 6      | 5    |
| Mesial tipping with buccal rotation  |                             |      |        |      |                         | 1    | 7      | 1    |
| Mesial tipping                       |                             |      |        |      |                         |      | 2      |      |
| Mesial moving                        | 2                           | 4    |        |      |                         |      | 2      | 2    |
| Distal moving                        |                             |      |        |      | 1                       |      |        |      |
| Distal moving with lingual rotation  |                             | 4    |        |      | 1                       | 3    | 1*     |      |
| Distal moving with buccal rotation   |                             |      |        |      |                         | 1*   | 1      | 1    |
| Lingual rotation                     |                             |      |        |      |                         |      |        | 3    |

\* The relation between the three measurements shows that tipping has occurred in these teeth.



TABLE VIII Showing the changes in widths of arches.

|                                                              | <u>Girls</u>   |              | <u>Boys</u>    |              |
|--------------------------------------------------------------|----------------|--------------|----------------|--------------|
|                                                              | <u>Younger</u> | <u>Older</u> | <u>Younger</u> | <u>Older</u> |
| <u>Upper anterior width:</u>                                 |                |              |                |              |
| Increase continuously or $\bar{c}$ fluctuations              | 2              | 2            | 10             | 6            |
| Decrease continuously or $\bar{c}$ fluctuations              |                | 1            | 1              | 2            |
| Increase after decrease                                      | 1              |              | 1              | 1            |
| Decrease after increase                                      | 2              | 1            |                | 1            |
| Slight changes or fluctuations                               | 1              | 2            |                |              |
| <u>Lower anterior width:</u>                                 |                |              |                |              |
| Increase continuously or $\bar{c}$ fluctuations              | 6              | 3            | 9              | 5            |
| Decrease continuously or $\bar{c}$ fluctuations              |                |              |                | 2            |
| Increase after decrease                                      |                |              | 2              | 2            |
| Decrease after increase                                      |                | 4            |                |              |
| Slight changes or fluctuations                               |                |              | 1              | 1            |
| <u>Width between upper 1st bicuspid or deciduous molars:</u> |                |              |                |              |
| Increase continuously                                        | 2              |              | 2              | 4            |
| Decrease continuously or $\bar{c}$ fluctuations              |                | 1            | 1              | 1            |
| Increase after decrease                                      |                | 2            |                |              |
| Decrease after increase                                      |                | 1            | 1              | 1            |
| Increase followed by fluctuations                            | 1              |              | 1              | 1            |
| Slight changes or fluctuations                               | 2              | 1            | 1              |              |
| <u>Width between lower 1st bicuspid or deciduous molars:</u> |                |              |                |              |
| Increase continuously                                        | 1              | 1            | 2              | 3            |
| Decrease continuously                                        |                | 1            |                | 1            |
| Increase after decrease                                      |                |              |                |              |
| Decrease after increase                                      |                | 1            | 3              |              |
| Slight changes or fluctuations                               | 2              |              |                |              |
| <u>Width between upper 2nd bicuspid or deciduous molars:</u> |                |              |                |              |
| Increase continuously                                        | 3              |              | 4              | 1            |
| Decrease continuously                                        |                |              |                | 1            |
| Increase after decrease                                      | 1              |              | 1              |              |
| Decrease after increase                                      |                |              |                | 1            |
| Slight changes or fluctuations                               |                | 1            | 1              |              |
| <u>Width between lower 2nd bicuspid or deciduous molars:</u> |                |              |                |              |
| Increase continuously                                        |                |              | 2              |              |
| Decrease after bicuspid are in                               | 1              |              |                |              |
| Slight changes or fluctuations                               |                | 1            | 1              |              |
| <u>Upper posterior width:</u>                                |                |              |                |              |
| Increase continuously or $\bar{c}$ fluctuations              | 3              | 2            | 6              | 4            |
| Decrease continuously or $\bar{c}$ fluctuations              |                |              |                | 2            |
| Decrease first then increase                                 | 2              | 2            | 4              |              |
| Slight changes or fluctuations                               | 1              | 3            | 2              | 4            |
| <u>Lower posterior width:</u>                                |                |              |                |              |
| Increase continuously or $\bar{c}$ fluctuations              |                | 3            | 2              |              |
| Decrease continuously or $\bar{c}$ fluctuations              | 1              |              | 4              | 4            |
| Increase after decrease                                      | 3              |              | 1              | 1            |
| Decrease after increase                                      | 1              |              |                | 1            |
| Slight changes or fluctuations                               | 1              | 3            | 5              | 4            |



## APPENDIX "P"

### Report to the Associate Committee on Dental Research

October, 1950

Subject: Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to Pablum history of the children's diet.

Grantee: M. Doreen Smith

Project No.: DR 13

Amount of Grant: \$1475.00

### SUMMARY OF WORK

#### Part I

The analysis of teeth extracted from Brantford children and sent to this department by Dr. H.K. Brown, Chief of the Dental Health Division, Ottawa, has been completed. The ratio of fluorine in dentin to fluorine in enamel was found to be 1.4 for the deciduous teeth as compared to 2.7 previously found with permanent teeth. Teeth from the Sarnia area are now on hand and analysis of these will proceed.

Contacts have been made with several Toronto dentists in an effort to collect teeth of children whose case histories relative to Pablum are known.

#### Part II

During most of the summer it has been impossible to do fluorine analyses because trouble was encountered with the method. A chemical method is used which requires that a determination blank be subtracted from the total fluorine found. It is essential, especially in samples of low fluorine content that a low stable blank be obtained. Thus when high variable blanks were found this problem had to be solved before accurate determinations could be made.

Possible sources of contamination were listed and each source examined in turn. The glassware itself, reagents used in cleaning the still, in distilling and in titrating were examined, as well as the fluorine fixative and the alkali used to neutralize the distillate. Atmospheric contamination due to fumes in the laboratory was also considered as a possibility. Poor results were found to be directly caused by contaminated alkali which was used to neutralize the distillate before carrying out the thorium nitrate titration. As yet the primary source of the contamination has not been learned.

"P-2" (Summary)

Although it occurred several times during a period when a new research procedure was being carried out in the department no proof of a connection between the new chemicals and the high blanks has been found. However, low stable blanks are again being obtained and regular analyses are now in procedure.

September 29th, 1950

"M. Doreen Smith"

Signature



# APPENDIX "P"

## REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to Pabulum history of the children's diets.

Grantee: M. Doreen Smith

Project No.: DR 13 Amount of Grant: \$1475.00

### Part I

#### Fluoride Content of Enamel and Dentin Fractions of Teeth Extracted from Brantford Children.

The analysis of Brantford teeth sent to this department by Dr. H.K. Brown, Chief of the Dental Health Division, Ottawa, has been completed. Since the last report several deciduous teeth have been analyzed and these are included in the table below.

#### Fluorine in Deciduous Brantford Teeth

| Name             | Age | Tooth Analyzed                   | Degree of Caries | Enamel % Fluorine | Dentin % Fluorine | Ratio of Dentin to Enamel |
|------------------|-----|----------------------------------|------------------|-------------------|-------------------|---------------------------|
| Robert W. Arthur | 5   | lower molar                      | none             | 0.022(2)          | .020(2)           | 0.9                       |
| Melvin Freedman  | 6   | lower molar                      | severe           | 0.0067            | .0093             | 1.4                       |
| Melvin Freedman  | 6   | pooled sample of 2 second molars | moderate         | 0.010(2)          | .018(8)           | 1.8                       |
| Selma Gasse      | 7   | pooled sample of two cuspids     | none             | 0.0018            | .0046             | 2.6                       |
| Donald Davies *  | 7   | pooled sample 2 lower 1st molars | severe           | 0.012(4)          | .018(1)           | 1.5                       |
| Thelma Minshall  | 9   | molar (crown only)               | moderate         | 0.0085            | .012(0)           | 1.4                       |

\* Analyzed since previous report.



Fluorine in Deciduous Brantford Teeth(Continued)

| Name             | Age | Tooth Analyzed                               | Degree of Caries | Enamel % Fluorine | Dentin % Fluorine | Ratio of Dentin to Enamel |
|------------------|-----|----------------------------------------------|------------------|-------------------|-------------------|---------------------------|
| Carol Allan*     | 10  | lower second molar                           | severe           | 0.013(5)          | .0096             | 0.7                       |
| Gerald Owens     | 10  | lower molar                                  | none             | 0.015(4)          | .014(3)           | 0.9                       |
| Robert Copeland* | 12  | pooled sample of upper & lower second molars | moderate         | 0.0075            | .010(2)           | 1.4                       |

\*Analyzed since previous report.

For this group of teeth the ratio of fluorine in dentin to fluorine in enamel has a mean of 1.4. The mean ratio for permanent teeth from Brantford children was 2.7(See previous report). This bears out the observation that the proportion of fluorine in dentin to enamel is greater in the permanent teeth than deciduous teeth.

Dr. Brown has recently sent us a number of teeth extracted from Sarnia children which have been cleaned and the caries removed in preparation for analysis. Sarnia is the control area in Dr. Brown's survey.

A number of contacts have been made with Toronto dentists in an effort to collect teeth from children whose case histories relative to Pabulum are known. However as yet few teeth which can be used have been collected.

## Part II

In the Department of Food Chemistry two projects have been carried on which necessitate fluorine analyses by the modified Willard and Winter method. The general method specifies an ashing of samples containing organic material with magnesium acetate as the F fixative, isolation of F by means of a distillation from perchloric acid and estimation in distillate by a thorium nitrate "back-titration" procedure.(1)

By careful choice of reagents and scrupulous cleansing of the distillation apparatus it is possible to obtain a low, stable determination blank. This is essential because with samples containing small amounts of F, this determination blank may represent a considerable part of the total F determined. During the three years that fluorine analyses have been made in this laboratory determination blanks have usually been from 3-4 micrograms of F with an increase to 6-7 micrograms when 1 ml. of 25% magnesium acetate solution was ashed in a Platinum dish as an F fixative.



"P-3"

Thus when high blanks and what was worse variable blanks were encountered in June, this problem had to be solved before accurate F determinations could be made. The results which were obtained in the following months will be given in tabular form with a brief explanation of what was done and why.

| Date    | "Still" used for Distillation | Conditions                                                                               | Amount of 0.05 N KOH for Neutralizing Distillate | Result Micro-grams | Comment                               |
|---------|-------------------------------|------------------------------------------------------------------------------------------|--------------------------------------------------|--------------------|---------------------------------------|
| June 2  | Still A(M.H.)                 | Blank-combination of contents from 2 Pt. dishes - 4ml. of 25% magnesium acetate fixative | 3.20 ml.                                         | 30.2               | High acid distillate and high results |
| June 6  | Still A(M.H.)                 | Blank- 2ml. of Mg. Acetate                                                               | 3.00                                             | 10.58              | Fairly good result                    |
| June 11 | Still A(M.H.)                 | Blank as of June 2                                                                       | 4.00                                             | -                  | High acid did not titrate             |
| June 11 | Still A(M.H.)                 | Recovery of 508 F                                                                        | 1.70                                             | 121.4%             | High recovery                         |
| June 12 | Still B(S.H.)                 | Blank-1 ml. Mg Acetate                                                                   | --                                               | 9.75               |                                       |

The determination blank can be minimized by preliminary treatment of the Still first by boiling 15-20 ml. of 171  $H_2SO_4$  to fumes and then by pouring off the acid and treating with hot 10% NaOH. At first it was believed that the high blanks were due to an inadequate cleaning of the Still. So as blanks were repeated the cleaning was done with extreme care. Occasionally a good result was obtained and high results were still obtained when the Still was cleaned as carefully as possible. Possible sources of contamination were listed and each possibility examined in turn-

Glassware

171  $H_2SO_4$  used in cleaning Still

10% NaOH used in cleaning Still

HClO<sub>4</sub> used in distillation

Silver sulphate used in distillation

p. nitrophenol indicator



0.05N KOH used to neutralize distillate  
 0.05N KCl used in titration  
 0.05N HCl used in titration  
 Hydroxylamine solution used in titration  
 Alizarin S indicator used in titration  
 Thorium nitrate solution  
 Fluorine free water  
 Mg Acetate fixative  
 Laboratory atmosphere itself may also have been contaminated.

To eliminate the possibility that an impure lot of magnesium acetate fixative was being used blanks were run with new lots of magnesium acetate. This did not improve the blank and it was also found that blanks without the fixative were likewise high. Next checked were the solutions used in the titration procedure. New KCl, Alizarin S, hydroxylamine and eventually HCl were all substituted. Results are tabulated as follows:

| Date    | "Still" used for Distillation | Conditions                                                  | KOH used for Neutralization | Result Micrograms | Comment                                                |
|---------|-------------------------------|-------------------------------------------------------------|-----------------------------|-------------------|--------------------------------------------------------|
| June 14 | Still B(S.H.)                 | Blank-1 ml. Mg acetate new Alizarin S and KCl for titration | 2.0 ml.                     | 13.5              | High results which often followed high acid distillate |
| June 15 | Still A(M.H.)                 | Blank-4 ml. Mg acetate new Mg acetate (used 2 Pt dishes)    | 1.30                        | 29.5              | High results with lower acid distillate                |
| June 15 | Still A(M.H.)                 | Blank-2 ml. Mg Acetate                                      | 1.90                        | 12.5              | Fairly high                                            |
| June 16 | Still A(M.H.)                 | Blank-no fixative                                           | 2.20                        | 7.5               | Usual apparatus blank is about 3-4                     |
| June 19 |                               | Same distillate as above titrated with new thorium nitrate  | -                           | 6.2               | Little difference in titration                         |
| June 20 | Still A(M.H.)                 | Blank without fixative & without $\text{Ag}_2(\text{SO}_4)$ | 2.00                        | 3.8               | Single titrations used Diff. of a few drops            |
|         |                               | Same distillate as above-new hydroxylamine                  |                             | 7.5               |                                                        |



"P-5"

| Date    | "Still" used for Distillation | Conditions                                                  | KOH used for Neutralization | Result Micrograms | Comment                                                               |
|---------|-------------------------------|-------------------------------------------------------------|-----------------------------|-------------------|-----------------------------------------------------------------------|
| June 20 | Still B(S.H.)                 | Blank-1 ml. Mg Acetate                                      | 1.50                        | 8.25              | Fair- slightly high                                                   |
| June 21 | Still A(M.H.)                 | Blank no fixative                                           | 2.40                        | 10.0              |                                                                       |
|         | Still A(M.H.)                 | Blank-4 ml. Mg Acetate                                      | 2.00                        | 29.5              | comparable to June 15                                                 |
|         | Still A(M.H.)                 | Recovery of 50 micrograms F                                 | 2.6                         | 91%               | low                                                                   |
| June 22 | Still B(S.H.)                 | Recovery of 50 micrograms of F (used Blank 8.25)            | 1.50                        | 96%               | Good recovery but depends on blank                                    |
| June 27 | Still B(S.H.)                 | Blank-1 ml. Mg Acetate                                      | 1.50                        | 8.50              | Fair blank                                                            |
|         | Still A(M.H.)                 | Blank no fixative                                           | 2.60                        | 24.5              | Very high and on same day                                             |
|         | Still A(M.H.)                 | Blank with 4 ml. Mg Acetate                                 | 2.00                        | 26.2              | A low blank was obtained. Mg Acetate gave little F increase           |
| June 28 | Still A(M.H.)                 | Blank with 2 ml. Mg Acetate                                 | 2.20                        | 30.0              |                                                                       |
| June 29 | Still B(M.H.)                 | Blank with 2 ml. Mg Acetate                                 | 2.00                        | 15.5              |                                                                       |
| June 30 | Still B(S.H.)                 | Blank no fixative new Perchloric acid & new silver sulphate | 3.00                        | 11.25             | Still high in spite of new $\text{HClO}_4$ & $\text{Ag}_2\text{SO}_4$ |
|         | Still A(M.H.)                 | Blank no fixative                                           | 2.40                        | 10.5              |                                                                       |
|         | Still A(M.H.)                 | Blank no fixative new silver sulphate                       | 2.00                        | 8.8               |                                                                       |



"P-6"

|               |                                     |      |      |                                                             |
|---------------|-------------------------------------|------|------|-------------------------------------------------------------|
| Still A(M.H.) | Blank no fixative<br>new Perchloric | 2.80 | 10.0 | New Perchloric<br>seems to con-<br>tribute sl.<br>high acid |
|---------------|-------------------------------------|------|------|-------------------------------------------------------------|

|        |               |                                                 |      |      |                         |
|--------|---------------|-------------------------------------------------|------|------|-------------------------|
| July 3 | Still A(M.H.) | Blank no fixative<br>without silver<br>sulphate | 3.30 | 13.8 | High acid<br>distillate |
|--------|---------------|-------------------------------------------------|------|------|-------------------------|

|               |                   |      |       |  |
|---------------|-------------------|------|-------|--|
| Still B(S.H.) | Blank no fixative | 2.00 | 11.75 |  |
|---------------|-------------------|------|-------|--|

|               |                   |      |       |           |
|---------------|-------------------|------|-------|-----------|
| Still B(S.H.) | Blank no fixative | 1.00 | 13.75 | Both high |
|---------------|-------------------|------|-------|-----------|

At this time it was decided that perhaps the distillation equipment had become contaminated and so a new Still (Still C) distilling head and condenser was tried. This did not lower the blank so the rubber joint which connected the distilling head and condenser was replaced by a ground glass joint to see if the rubber had been contributing appreciable F. Again no difference was found.

|        |               |                                                |     |      |            |
|--------|---------------|------------------------------------------------|-----|------|------------|
| July 4 | Still C(M.H.) | Blank-new still<br>& distillation<br>apparatus | 1.8 | 12.5 | Blank high |
|--------|---------------|------------------------------------------------|-----|------|------------|

|        |               |                                 |     |     |                                             |
|--------|---------------|---------------------------------|-----|-----|---------------------------------------------|
| July 5 | Still C(M.H.) | Blank-no fixa-<br>tive as above | 1.2 | 8.2 | Blank slightly<br>lower as Still<br>is used |
|--------|---------------|---------------------------------|-----|-----|---------------------------------------------|

|   |                                                                |  |     |                            |
|---|----------------------------------------------------------------|--|-----|----------------------------|
| - | Distillate from<br>above-new<br>$K_2SiF_6$ sol'n<br>from solid |  | 8.2 | $K_2SiF_6$ not at<br>fault |
|---|----------------------------------------------------------------|--|-----|----------------------------|

|        |               |                                                                                  |      |       |                                                                 |
|--------|---------------|----------------------------------------------------------------------------------|------|-------|-----------------------------------------------------------------|
| July 7 | Still B(S.H.) | Blank -1 ml. Mg<br>Acetate new<br>ground glass<br>joint To elim-<br>inate rubber | 2.00 | 16.25 | High- partly<br>because of<br>longer time<br>taken to<br>distil |
|--------|---------------|----------------------------------------------------------------------------------|------|-------|-----------------------------------------------------------------|

|               |                        |      |                                         |
|---------------|------------------------|------|-----------------------------------------|
| Still B(S.H.) | Blank-no fixa-<br>tive | 5.00 | High acid distillate<br>Did not titrate |
|---------------|------------------------|------|-----------------------------------------|

New 0.05N KOH was made up for neutralizing the distillate and at the same time 0.05N HCl was also used up and had to be made up fresh. Two results on July 11 using both new KOH and HCl were found to be low.



|         |               |                        |      |      |           |
|---------|---------------|------------------------|------|------|-----------|
| July 11 | Still B(S.H.) | Blank no fixative      | 1.00 | 3.75 | Good      |
|         | Still B(S.H.) | Blank-1 ml. Mg Acetate | 1.00 | 6.75 | Also good |

As it later turned out, the KOH somehow became contaminated with F. It did not give a high blank in these two runs so presumably it was not contaminated when first made up. However blanks determined about one week later were again high.

|         |               |                        |      |       |            |
|---------|---------------|------------------------|------|-------|------------|
| July 17 | Still B(S.H.) | Blank no fixative      | 1.50 | 8.50  | High again |
|         | Still B(S.H.) | Blank-1 ml. Mg Acetate | 1.50 | 11.25 |            |

|         |               |                   |      |      |                                      |
|---------|---------------|-------------------|------|------|--------------------------------------|
| July 20 | Still B(S.H.) | Blank no fixative | 2.00 | 8.75 | Higher acid followed by high results |
|---------|---------------|-------------------|------|------|--------------------------------------|

Another possible source of contamination is atmospheric fumes and about this time interest was aroused in an organic chemical that was also being used in the research laboratory. This chemical ethylene diamine was known to be unstable in the presence of water vapour and  $\text{CO}_2$ . Experiments shown below seemed to indicate that it was involved - though in what manner it was impossible to say.

|         |               |                                                                                       |      |      |                   |
|---------|---------------|---------------------------------------------------------------------------------------|------|------|-------------------|
| July 24 | Still C(S.H.) | Blank no fixative new 10% NaOH used for cleaning. E.D. Had not been used for 3-4 days | 1.00 | 6.00 | Fairly good blank |
|---------|---------------|---------------------------------------------------------------------------------------|------|------|-------------------|

|         |               |                                            |      |       |  |
|---------|---------------|--------------------------------------------|------|-------|--|
| July 24 | Still C(S.H.) | As above except that 1 ml. Mg Acetate used | 1.50 | 10.50 |  |
|---------|---------------|--------------------------------------------|------|-------|--|

|         |               |                   |      |      |                                           |
|---------|---------------|-------------------|------|------|-------------------------------------------|
| July 25 | Still C(S.H.) | Blank no fixative | 1.00 | 6.00 | At this time 6.00 was the low for Still C |
|---------|---------------|-------------------|------|------|-------------------------------------------|



|         |               |                                                                |                                              |       |                                                   |
|---------|---------------|----------------------------------------------------------------|----------------------------------------------|-------|---------------------------------------------------|
| July 26 | Still C(S.H.) | Blank no fixative. Ethylene diamine was used in same lab.      | 1.50                                         | 11.75 | Higher blank when E.D. used                       |
|         | Still C(S.H.) | Repeated in aft. only residual E.D. from A.M. present          | 1.00                                         | 7.00  |                                                   |
| July 27 | Still C(S.H.) | Exposed beaker of E.D. while distilling blank without fixative | 3.5                                          | 21.75 | High acid and high blank                          |
| July 28 | Still C(S.H.) | Blank no fixative only E.D. present must be residual           | 3.0                                          | 20.25 | Sl. less acid distillate and blank a little lower |
| Aug. 1  | Still C(S.H.) | Blank no fixative - no fresh E.D.                              | 1.00                                         | 6.00  | Higher blank follows high acid                    |
|         | Still D(S.H.) | As above new Still                                             | 1.80                                         | 13.00 |                                                   |
| Aug. 2  | Still C(S.H.) | Blank no fixative                                              | High acid distillate (6.00+) Did not titrate |       |                                                   |
|         | Still D(S.H.) | As above E.D. used in lab several doors away                   | 1.50                                         | 13.25 |                                                   |
| Aug. 3  | Still C(S.H.) | Blank no fixative. E.D. not used in bldg.                      | 1.00                                         | 7.50  | Slightly lower than previous days                 |
|         | Still D(S.H.) |                                                                | 1.50                                         | 12.50 |                                                   |
| Aug. 4  | Still C(S.H.) | Blank no fixative E.D. used in another lab                     | 1.00                                         | 8.00  |                                                   |
|         | Still D(S.H.) |                                                                | 2.50                                         | 22.0  |                                                   |



|        |               |                                                 |      |       |                        |
|--------|---------------|-------------------------------------------------|------|-------|------------------------|
| Aug. 8 | Still D(S.H.) | Blank no fixative E.D. not used for 3 days      | 1.00 | 8.75  | Blanks slightly lower  |
|        | Still C(M.H.) |                                                 | 1.00 | 7.50  |                        |
| Aug. 9 | Still D(S.H.) | Blank no fixative E.D. used in neighbouring lab | 1.50 | 10.75 | Higher blanks obtained |
|        | Still C(M.H.) |                                                 | 1.50 | 20.0  |                        |

No further work was done on this problem until ethylene diamine was no longer being used in the labs. A series of blanks were now found to be higher than ever.

|         |               |                   |      |      |                                                     |
|---------|---------------|-------------------|------|------|-----------------------------------------------------|
| Aug. 22 | Still C(M.H.) | Blank no fixative | 2.50 | 32.5 |                                                     |
| Aug. 23 | Still C(M.H.) | "                 | 1.5  | 21.2 | The higher acid the distillate the higher the blank |
|         | Still C(M.H.) | "                 | 1.2  | 17.5 |                                                     |
| Aug. 24 | Still C(M.H.) | "                 | 2.0  | 26.2 |                                                     |
| Aug. 25 | Still C(M.H.) | "                 | 2.50 | 30.0 |                                                     |

In order to see if the atmosphere of the research lab was contaminated, the apparatus was moved to different parts of the building, then entirely new apparatus was used and again different reagents were substituted.

|         |               |                                                              |      |      |                           |
|---------|---------------|--------------------------------------------------------------|------|------|---------------------------|
| Aug. 25 | Still C(M.H.) | Blank no fixative Lab No. 314                                | 2.50 | 35.0 | Very high                 |
| Aug. 28 | Still C(M.H.) | Blank-same Perchloric as above, new $\text{Ag}_2\text{SO}_4$ | 1.20 | 15.0 | Acidity and F both lower  |
| Sept. 1 | Still C(M.H.) | Blank-new Perchloric(Bakers), in Textile Lab No.311          | 4.00 |      | High acid did not titrate |



|          |               |                                                |     |      |                                 |
|----------|---------------|------------------------------------------------|-----|------|---------------------------------|
| Sept. 5  | Still C(M.H.) | As above omitting preliminary cleaning         | 2.0 | 27.5 |                                 |
| Sept. 8  | Still C(M.H.) | Blank-in basement. New p-nitrophenol indicator | 2.7 | 40.0 | High                            |
| Sept. 11 | Still C(M.H.) | Blank-in basement. New Perchloric (G.F. Smith) | 3.4 |      | High did not complete titration |
| Sept. 11 | Still C(M.H.) | Blank-in basement Merck Perchloric             | 1.5 | 27.5 |                                 |
| Sept. 12 | Still D(S.H.) | Blank in original lab-G.F. Smith perchloric    | 3.4 |      | High result not titratable      |
| Sept. 13 | Still C(M.H.) | Blank-using doubly boiled Perchloric           | 1.0 | 16.2 |                                 |

Since high results seemed to follow the use of considerable KOH to neutralize high acid distillates it was decided to make up fresh KOH including a new 50% KOH stock solution. Using fresh 0.05N KOH low blanks in the normal range were obtained.

|          |               |                                                 |      |       |                                              |
|----------|---------------|-------------------------------------------------|------|-------|----------------------------------------------|
| Sept. 13 | Still C(M.H.) | Blank no fixative Old $\text{HClO}_4$ (Baker's) | 2.8  | 1.25  | High acid distillate followed by low results |
| Sept. 14 | Still D(S.H.) | Blank as above                                  | 1.40 | 3.75  |                                              |
| Sept. 15 | Still D(S.H.) | Analyzed 5 ml. of old 50% KOH (Stock)           | 1.70 | 28.75 | Slightly contaminated                        |
| Sept. 16 | Still C(S.H.) | Blank no fixative                               | 1.10 | 2.75  | Low blank                                    |
| Sept. 16 | Still D(S.H.) | Analyzed 5 ml. new 0.05N KOH                    | 1.70 | 2.25  | Contamination nil                            |
| Sept. 18 | Still C(M.H.) | Analyzed 5 ml. old 0.05N KOH                    | 1.20 | 81.75 | Highly contaminated                          |

NOTE: M.H. . Mary Ham and S.H. Shirley Harness



It now appeared that the high blanks were chiefly due to contaminated alkali although the possibility that other reagents had contributed especially at first was not entirely discounted. The chief problem remaining was what had contaminated the alkali as two successive lots had been contaminated. Although the stock solution (50% KOH) was also contaminated it did not contain enough F to make possible the contamination found in the 0.05N KOH. It did not seem possible that this F could have been introduced by pipettes since an F solution is rarely touched by pipettes and also 3 different solutions had been contaminated.

Another possibility was atmospheric contamination from fumes in the lab and this is still considered the most likely source although it has not yet been proved. A series of experiments were carried out designed to see whether ethylene diamine under a variety of conditions was capable of contaminating the alkali. No positive results have been obtained and analysis of ethylene diamine itself showed that it contains very little F.

High acid distillates were encountered in this investigation occurring for no apparent reason. Neutralization of such a distillate with contaminated alkali would automatically give a high blank value. Although the 0.05N KOH solution has been shown to be directly responsible for most of the poor results, the source of its contamination still remains unknown.

" M. Doreen Smith "





## APPENDIX "Q"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches.

Grantee: Dr. Norma Ford Walker

Project: DR 15

#### PREFACE

The aim of the study has been to determine whether or not irregularity in the growth of the jaws (which may be observed in the breadth changes of the dental arches) may be detected as early as the time of the eruption of the deciduous teeth and whether or not there is an association between them. Of primary importance therefore has been the establishing of a pattern representing a reliable norm for the eruption of these first teeth, to which end the eruption records of 360 individuals have been collected and the time, in days, for the eruption of each tooth calculated; and secondly, acquiring accurate measurements of the dental arches in an attempt to observe rates of growth and consequent development. For the latter purpose series of cases (an average of 4 sets in each series) of 112 individuals have been assembled. The breadth of each cast has been measured in three different locations. These two basic sets of data have provided the opportunity of making various analyses.

This is the first time that an attempt has been made to establish an association between the pattern of eruption of the deciduous teeth and the rates of growth of the dental arches.

#### SUMMARY OF WORK

##### I Analysis of the eruptive time of the teeth

1. Comparison of the eruptive pattern of a random group of boys with a random group of girls. The mean eruptive time ( $\bar{X}$ ) in days, the standard deviation ( $S$ ), the standard error ( $S_{\bar{X}}$ ) and the standard error of the difference ( $S_{diff}$ ), the sexes separated, constituted the first calculations. From these statistics it is possible to show that no significant difference exists in the eruptive time of the deciduous teeth of a random group of boys and girls.

2. From further statistics it has been observed that no true difference exists between the eruptive time for twins and singletons in each of the sex categories (although the prenatal environment of a twin birth is known to be more unfavourable than that of a single birth).



3. The similar environment of fraternal twins appears to be a factor of little significance in its effect on the eruptive pattern.

4. Random groups with different hereditary factors show far greater difference in eruptive times (expressed in the standard deviation) than groups with the same hereditary factors.

5. The coefficient of variability determined from the random sample of boys and girls shows the incisors to be relatively more variable than the cuspids or molars.

As a result of the foregoing analysis it has been possible to establish an eruptive pattern (one for each sex) representative of the population. The eruption of each individual making up the sample has been compared with this mean eruptive time. The relation existing between the eruption of the individual and the mean eruptive time formed the basis for determining the type of eruptive pattern.

## II Determining normality in eruptive pattern

Of the original sample of eruption records of 360 children and of dental casts of 112 children there were 90 individuals who had both complete eruptions (except for 16 who lacked the eruptive time of the second molars) and series of casts. When the eruption of each of these 90 individuals is compared with the mean eruptive time certain differences appear. It was found profitable to put each individual in one of two categories. Category A was comprised of individuals with regular eruption, Category B those with irregular eruption.

### 1. Category A. Eruptions showing regularity

Regularity of eruption has been compared to development as propounded by Wetzel who considers that regularity is indicative of normal development. Regularity in eruption was believed to be demonstrated by those cases in which there was conformity to the usual pattern of eruption as well as consistency in time of eruption. With respect to the mean eruptive time a child may be close, early or late, which timing is set with the eruption of the first two teeth. Regularity which has been taken to mean normality is expressed in a child's ability to keep to this initial timing with the result that he remains in his own channel of eruption whether it be late, close, or early with respect to the mean eruptive time.

Sample A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, which are appended show the three types of regular eruption viz.:

(A<sub>1</sub>)a. Eruptions that are consistently close to the mean eruptive time.

(A<sub>3</sub>)b. Eruptions that are consistently early with respect to the mean eruptive time.

(A<sub>2</sub>)c. Eruptions that are consistently late with respect to the mean eruptive time.



2. Category B. Eruptions showing Irregularity.

Samples B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and B<sub>4</sub> which are appended serve to illustrate the types of eruptions regarded as irregular. In these cases there appears to have been no preferred channel of eruption.

Sample B<sub>1</sub>. Mixed eruption (M). Irregularity in these cases is exhibited within the pattern. These individuals start and finish eruption with some uniformity, but during eruption the original timing varies to an extreme.

Sample B<sub>2</sub>. Eruptions starting early with respect to the mean eruptive time and finishing late. (SE.FL).

Sample B<sub>3</sub>. Eruptions starting close to the mean eruptive time, but an increase in initial pace results in an early finish with respect to the mean eruptive time. (SM.FE)

Sample B<sub>4</sub>. Eruptions starting late with respect to the mean eruptive time concluding with the precocious appearance of the last teeth. (SL.FE.)

III Determining Normality in size of dental arch

As in the case of the eruptive pattern, stencils were used for determining normality in size of the dental arches, these stencils being based on the results of Lewis and Lehman (1929). These results comprise mean values, standard deviation, and standard error, for measurements at C|C, D|D, E|E, and U|U, D|D, and E|E, and were based on a group of 170 children ranging in age from 18 mos. to 9 1/2 years. Measurements of the dental arches of each individual in our study when compared with this mean measurement revealed variations. As in the case of the eruptive pattern it was found profitable to put each individual into one of two categories. Category a, comprised of individuals exemplifying regular arch development, Category b, those showing irregularity.

1. Category a, Individuals with regular dental arch development

1. Cases in which all three measurements of upper and lower arch lay either close to the mean measurement or were all either on the upper or on the lower side of the mean measurement within the standard deviation. Sample a<sub>1</sub>.

2. Cases in which all three measurements of upper and lower arch lay outside the standard deviation on the upper side of the mean measurement. Sample a<sub>2</sub>.



2. Category b. Individuals with irregularity in the development of the dental arches.

1. Cases in which all three measurements of one arch approach the mean measurement or lie within the standard deviation on one side of the mean while the three measurements of the other arch lie outside the standard deviation on the opposite side of the mean.

Sample b1.

2. Those cases in which the irregularity is not expressed in all three measurements of one or other arch but is restricted to one or perhaps two measurements in one or other arch or in both arches at different localities. Sample b2.

IV Factors affecting eruption and rates of growth of the dental arches

Heredity. (Discussed in thesis)

Diet. (Discussed in thesis)

Sucking Habits. (Discussed more fully in thesis) The results of this study have shown that sucking habits may or may not be associated with irregular development of the dental arches. Of a group of 11 pairs of monozygotic twins, 5 contained at least one member who was a continuous sucker. Of these 5 pairs, there were 2 sets in which there was only one sucker, and in one case the sucker showed regular development, in the other the sucker showed irregular development. Of 2 sets in which both were suckers neither showed irregularity in development. Of one set in which both sucked, the more determined sucker showed regularity of development. Finally in one pair in which neither sucked, one child showed irregular development. It would seem that factors other than sucking are influential in the development of the dental arches. (Table I appended).

V Association between eruptive pattern and rates of growth of the dental arches

Analysis 1. The total sample of 90 cases were considered individually for type of eruption and dental arch development. The distribution of the data arranged in a contingency table was as follows:

|                                                                        |    |
|------------------------------------------------------------------------|----|
| Individuals showing <u>concordance</u> with respect to both processes: |    |
| Regular eruption and regular dental arch development.....              | 31 |
| Irregular eruption and irregular dental arch development....           | 34 |

|                                                                 |    |
|-----------------------------------------------------------------|----|
| Individuals showing discordance with respect to both processes: |    |
| Regular eruption and irregular dental arch development.....     | 10 |
| Irregular eruption and regular dental arch development.....     | 15 |

$$\chi^2 = 18.089$$
$$P = < .01$$



Analysis 2. In the second analysis, a pair of monozygotic twins was counted as a single individual and in addition all individuals who were suckers for at least 4 years and/or were on special diets were excluded. The total number of individuals in the sample was consequently reduced to 49. In this analysis the chi-square value was 10.864,  $P = < .01$ . It appeared therefore in analysis 2 as in analysis 1 that the association between eruption and development of the dental arches was not one of chance. The third analysis was made to determine whether or not an association exists between eruption and type of development in a random group of non-related singletons. The total number of singletons was 33 but of these 11 were either suckers and/or had had a special diet for some time thus reducing the number to 22. The contingency table for these unrelated individuals was as follows:

Concordance.

Individuals with regular eruption and regular development of the dental arches ..... 9

Individuals with irregular eruption and irregular development of the arches ..... 10

Discordance.

Individuals with regular eruption and irregular dental arch development ..... 1

Individuals with irregular eruption and regular dental arch development ..... 2

$$\chi^2 = 11.735$$

$$P = < .01$$

Although a sample made up of 22 individuals is a small sample nevertheless the results obtained from this analysis showed that when the sample contained no related individuals and no twins which might have influenced the distribution, the association between type of eruption and the development of the dental arches is still not one of chance. These results indicate that the probability for such distributions between patterns of eruption and growth of the dental arches occurring by chance alone is less than one in one hundred. It may therefore be concluded that there is an association between the patterns of eruption and the growth of the dental arches. Both processes appear to be governed by the same factor or factors.

VI Frequency of irregularity expressed in certain groups of teeth.

(Discussed in thesis.)



TABLE I Showing the effect of sucking habits on development of the dental arches in MZ twins.

(All showed concordance in the type of eruption.)

| Number of Pairs | Twin A           | Twin B                   | ARCH DEVELOPMENT     |                      |
|-----------------|------------------|--------------------------|----------------------|----------------------|
|                 |                  |                          | Twin A               | Twin B               |
| 2*              | Sucker<br>Sucker | Non-sucker<br>Non-sucker | Regular<br>Irregular | Irregular<br>Regular |
| 2               | Sucker<br>Sucker | Sucker<br>Sucker         | Regular<br>Regular   | Regular<br>Regular   |
| 1               | Sucker           | Sucker                   | Regular              | Irregular            |
| 1               | Non-sucker       | Non-sucker               | Irregular            | Regular              |
| 2               | Non-sucker       | Non-sucker               | Irregular            | Irregular            |
| 2               | Non-sucker       | Non-sucker               | Regular              | Regular              |
| 1*              | Non-sucker       | Non-sucker               | Regular              | Regular              |

\* Children with incomplete eruptive records



DAYS

SAMPLE A<sub>1</sub> Eruption is consistently close to the mean eruptive time.

Graph showing the mean eruptive time in days for the deciduous teeth of female children.

Solid line: mean eruptive time  
Broken line: eruption of the individual.  
Corresponding pairs of teeth are connected.

900

860

820

780

740

700

660

620

580

540

500

460

420

380

340

330

260

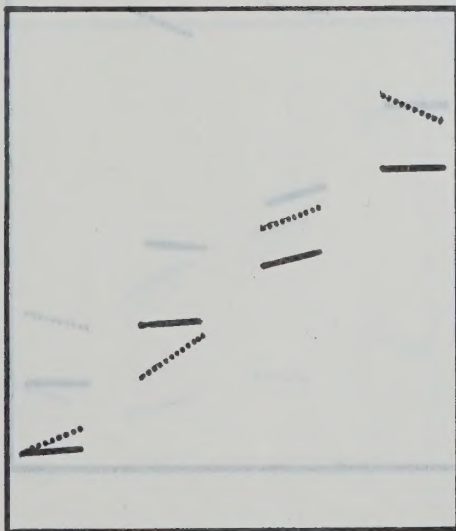
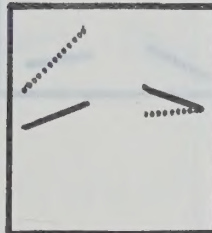
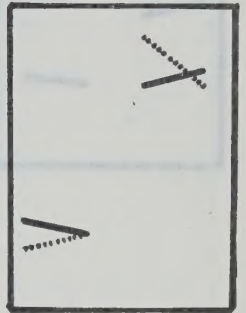
220

180

100

LICI ULCI ULLI LLLI ULFM LLFM ULC LLC LLSM ULSM  
LRCI URCI URLI LRLI URFM LRFM URC LRC LRSM URSM

20 deciduous teeth.







DAYS

SAMPLE A<sub>2</sub> Eruption is consistently late with respect to the mean eruptive time.

Graph showing the mean eruptive time in days for the deciduous teeth of female children.

Solid line = mean eruptive time

Broken line = eruption of the individual.

Corresponding pairs of teeth are connected.

900

860

800

760

740

700

660

620

580

540

500

460

420

380

340

300

260

220

180

140

100

LLCI ULCI ULLI LLLI ULFI LLFI ULC LLC LLSM ULSM  
LRCI URCI URLI LRLI URFI LRFI URC LRC LRSM URSM  
20 deciduous teeth.





DAYS

SAMPLE A

3. Eruption is consistently early with respect to the mean eruptive time.

Graph showing the mean eruptive time in days for the deciduous teeth of male children.

Solid line: mean eruptive time  
Broken line: eruption of the individual  
Corresponding pairs of teeth are connected.

860

820

780

740

700

660

620

580

540

500

460

420

380

340

300

260

220

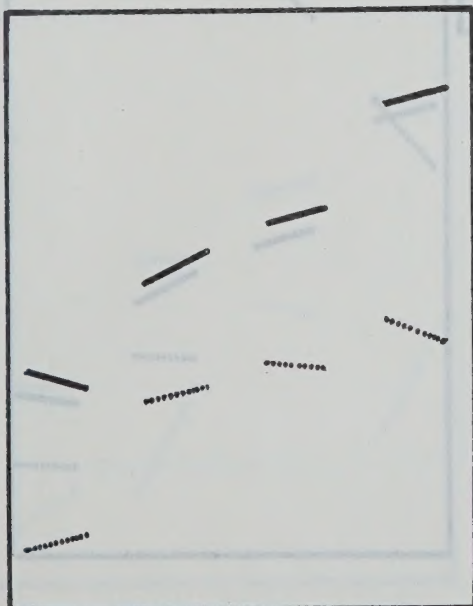
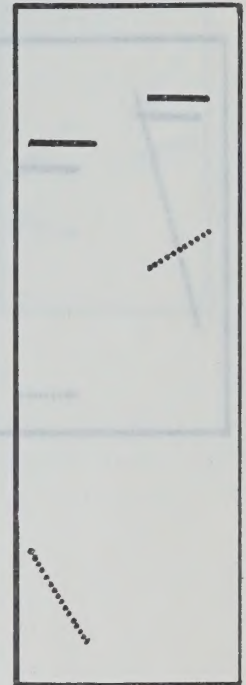
180

140

100

LLCI ULCI ULLI LLLI ULFM LLEM ULC LLC LLSM ULSM  
LRCI URCL URLLI LRLLI URFM LRFM URC LRC LRSM URSM

20 deciduous teeth.







"Q-10"

SAMPLE B<sub>1</sub> Mixed eruption.

Graph showing the mean eruptive time in days for the deciduous teeth of male children.

Solid line: mean eruptive time

Broken line: eruption of the individual.

Corresponding pairs of teeth are joined.

DAYS

860

820

780

740

700

660

620

580

540

500

460

420

380

340

300

260

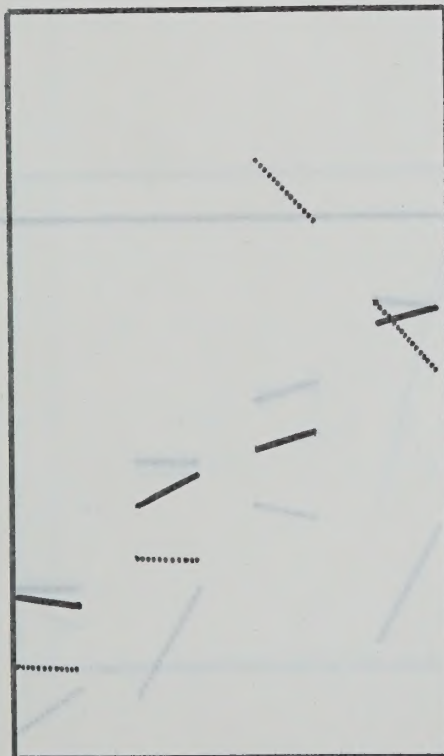
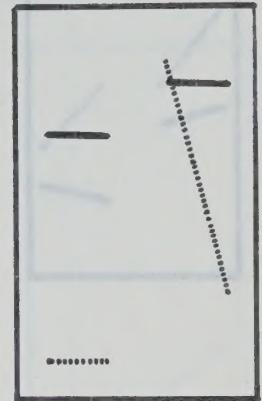
220

180

140

100

LLCI ULCI ULLI LLLI ULFM LLFM ULC LLC LLSM ULSM  
LRCI URCI URLI LRLI URFM LRFM URC LRC LRSM URSM  
20 deciduous teeth.







"Q-11"

DAYS

SAMPLE B<sub>2</sub> Eruption starts early with respect to the mean eruptive time, finishes late.

Graph showing the mean eruptive in days for the deciduous teeth of female children.  
Solid line: mean eruptive time  
Broken line: eruption of the individual.  
Corresponding pairs of teeth are joined.

860

820

780

740

720

660

620

580

540

500

460

420

380

340

300

260

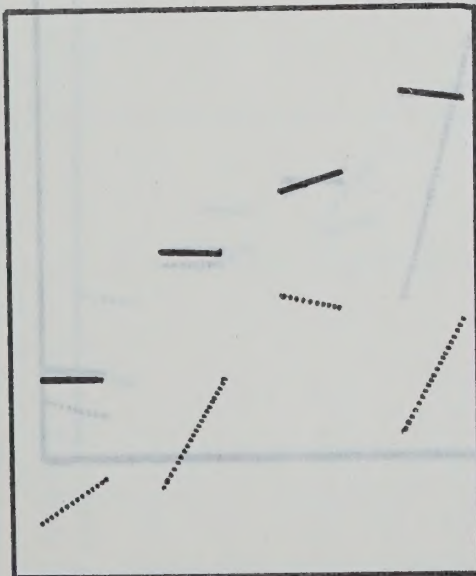
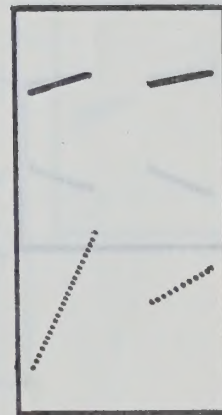
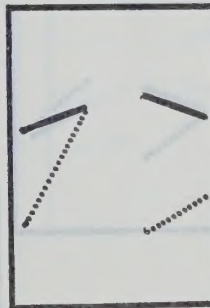
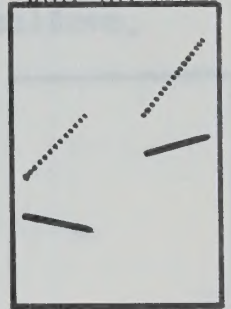
220

180

140

100

LLCI ULCI ULLI LLLI ULFM LLFM ULC LLC LLSM ULSM  
LRCL URCI URLI LRLL URFM LRFM URC LRC LRSM UREM  
20 deciduous teeth.







"Q-12"

DAYS

SAMPLE B<sub>3</sub> Eruption starts close to the mean eruptive time, finishes early.

Graph showing the mean eruptive time in days for the deciduous teeth of female children.

Solid line = mean eruptive time  
Broken line = eruption of the individual.  
Corresponding pairs of teeth are connected.

860

820

780

740

700

660

620

580

540

500

460

420

380

340

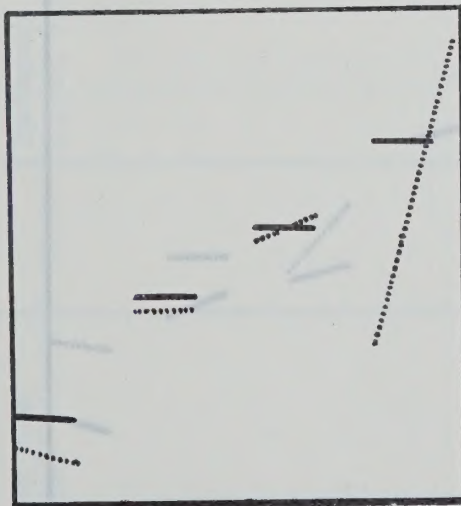
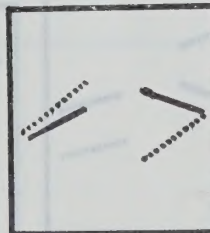
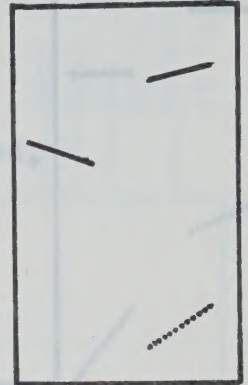
300

260

220

180

140



100 LLCI ULCI ULLI LLLI ULFM LLFM ULC LLC LLSM ULSM  
LRCI URCI URLI LRLI URFM LRFM URC LRC LRSM URSM

20 deciduous teeth.





"Q-13"

DAYS

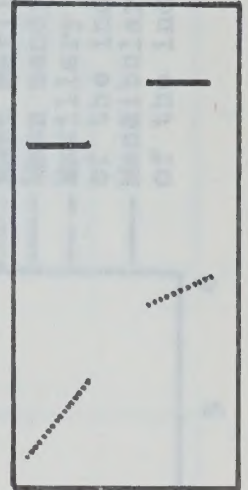
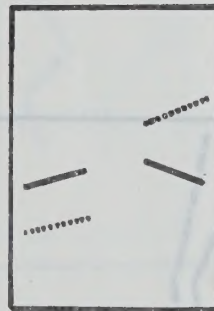
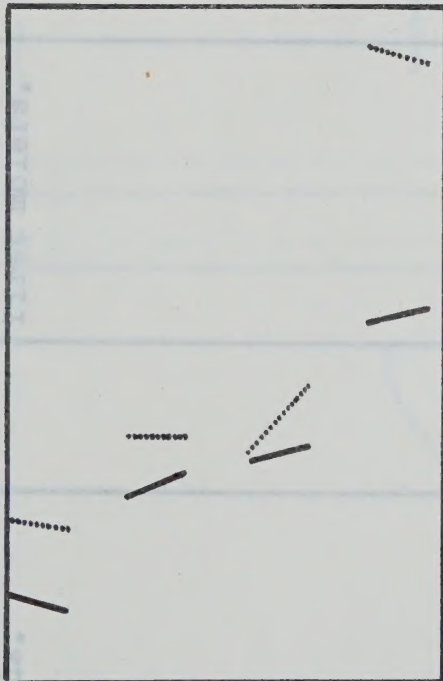
SAMPLE B4 Eruption starts late with respect to the mean eruptive time, finishes early.

Graph showing the mean eruptive time in days for the deciduous teeth of male children.

Solid line: mean eruptive time  
Broken line: eruption of the individual.  
Corresponding pairs of teeth are connected.

860  
820  
780  
740  
700  
660  
620  
580  
540  
500  
460  
420  
380  
340  
300  
260  
220  
180  
140  
100

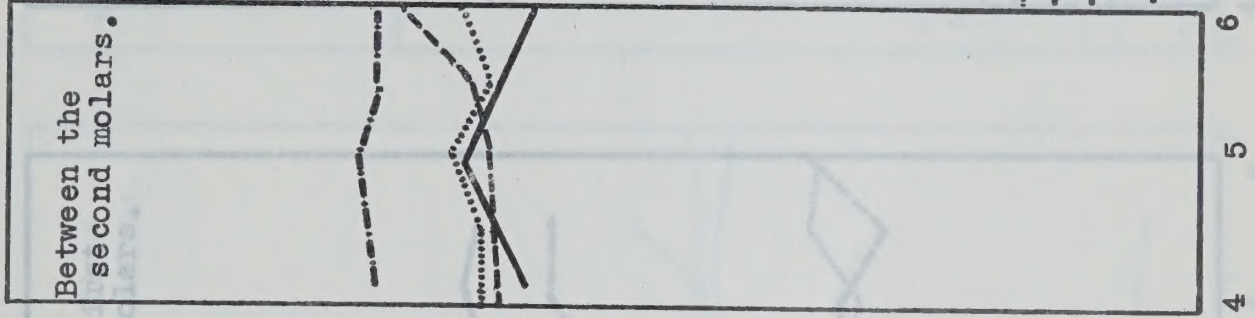
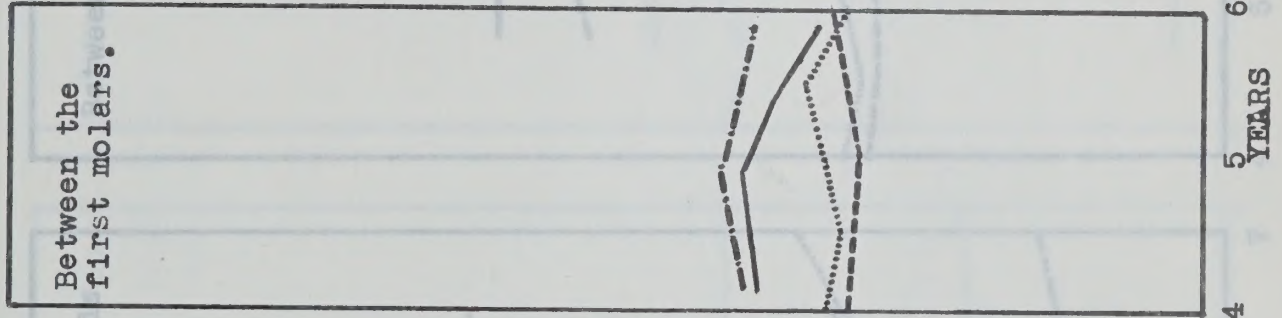
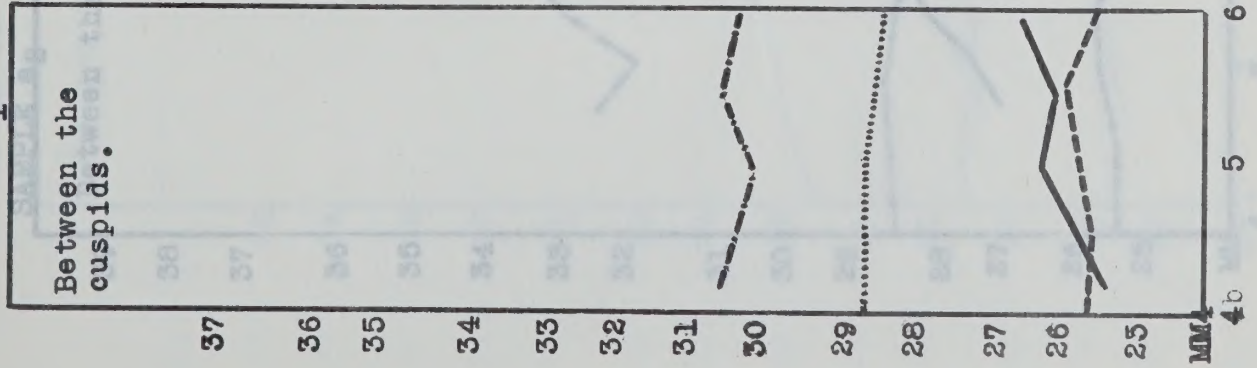
LLCI ULCI ULLI LLLI ULFM LLFM ULC LLC LLSM ULSM  
LRCI URCI URLI LRLI URFM LRFM URC LRC LRSM URSM  
20 deciduous teeth.







SAMPLE a<sub>1</sub>



Mean widths of the dental arches compiled by Lewis and Lehman-1929

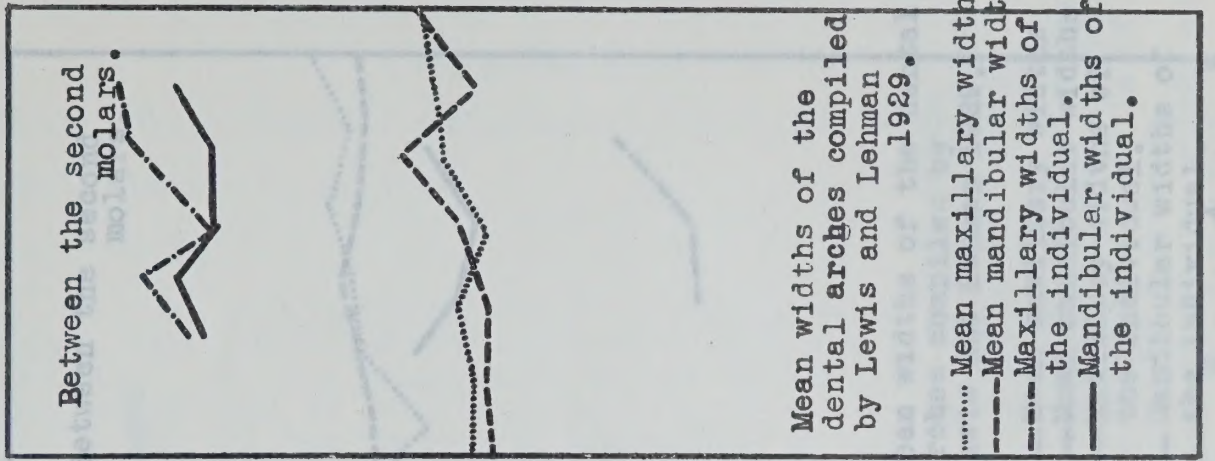
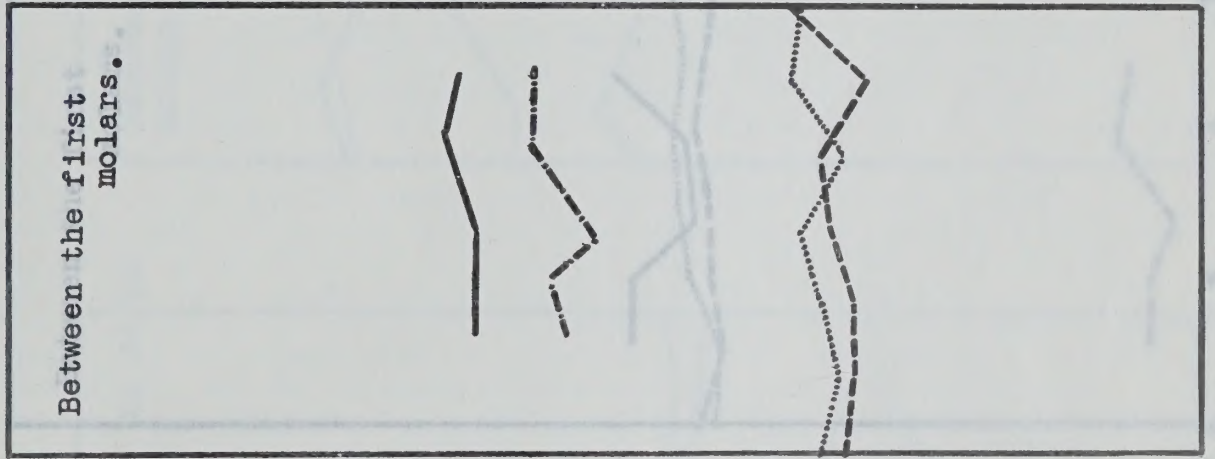
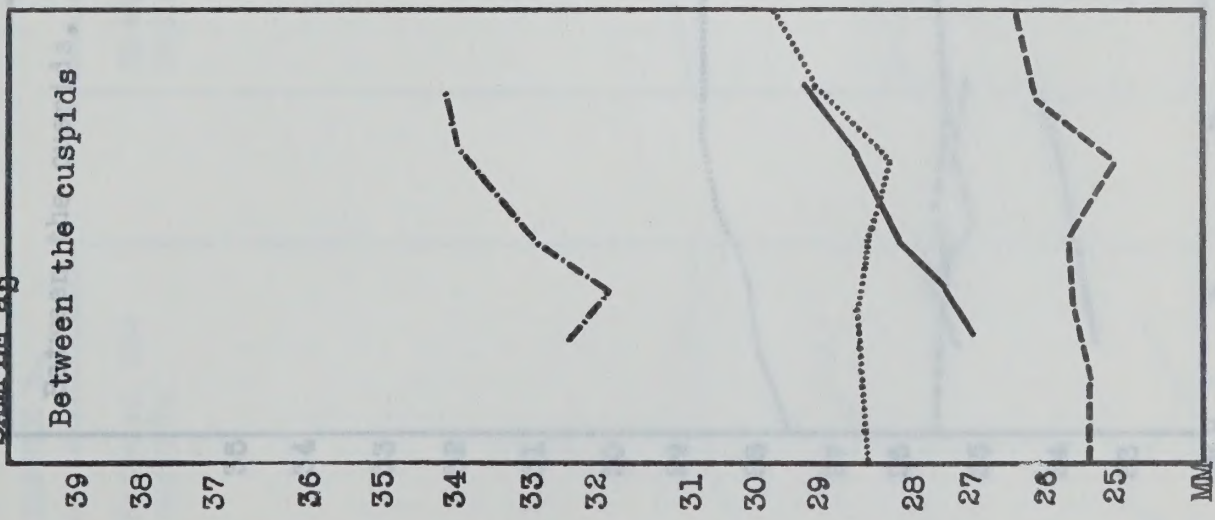
..... Mean maxillary widths  
 ----- Mean mandibular "  
 -.-.-.-. Maxillary widths  
 of the individual  
 — Mandibular widths  
 of the individual.





"Q-15" Q-16"

SAMPLE b1  
SAMPLE a9



Mean widths of the dental arches compiled by Lewis and Lehman 1929.

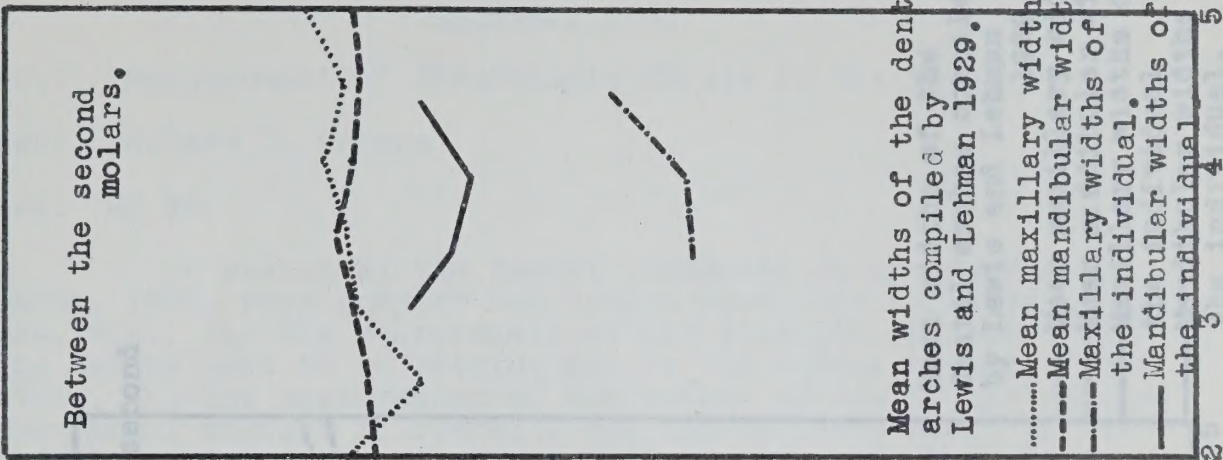
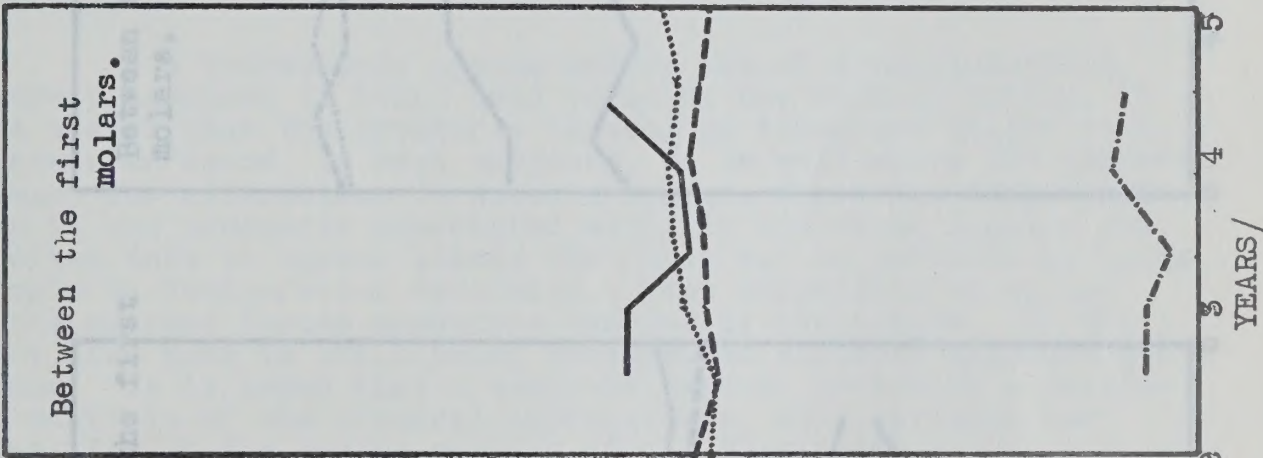
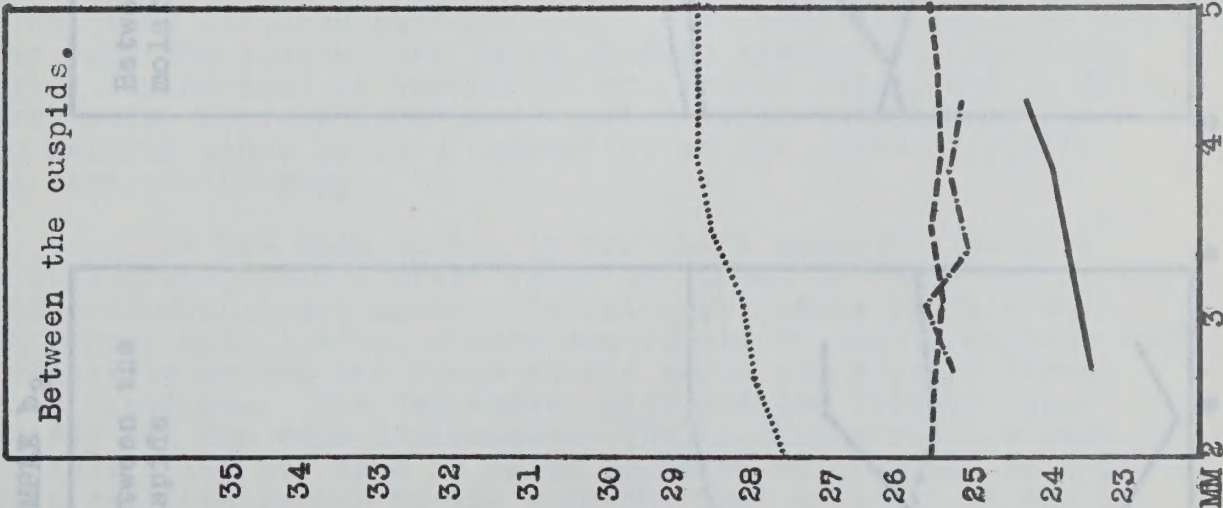
..... Mean maxillary widths  
----- Mean mandibular widths  
----- Maxillary widths of the individual.  
----- Mandibular widths of the individual.

MM 4 5 6 7 YEARS 4 5 6 7





SAMPLE  $b_1$



Mean widths of the dental arches compiled by Lewis and Lehman 1929.

..... Mean maxillary widths  
 ----- Mean mandibular widths  
 - - - - - Maxillary widths of the individual.  
 ————— Mandibular widths of the individual.

YEARS/





# REPORT TO THE ASSOCIATE COMMISSIONER ON DENTAL RESEARCH

October, 1950

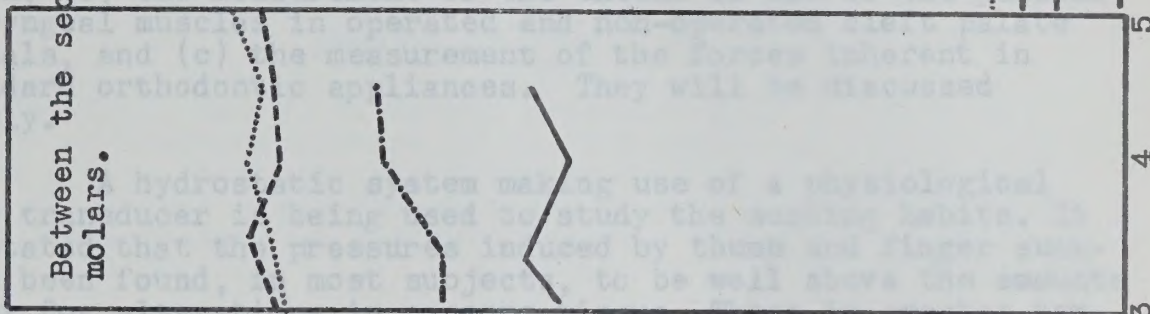
Subject: Measurement of Physiologic Forces in the

Grantee: Robert E. Moyers

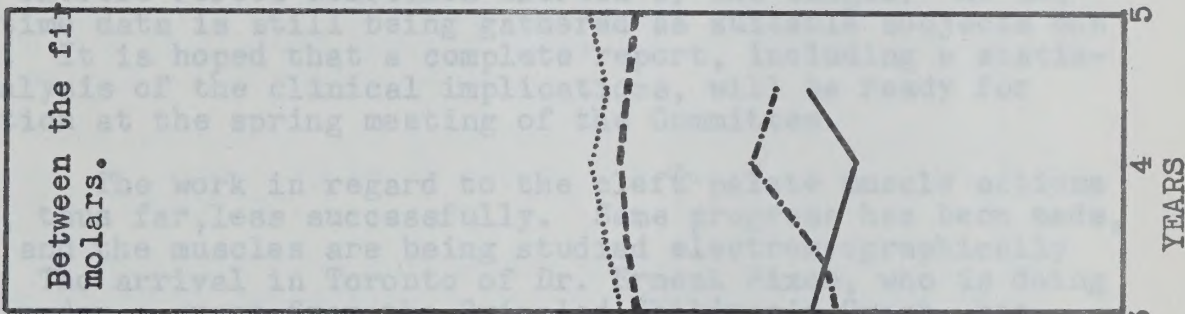
Project: DR 26

Mean widths of the dental arches compiled by Lewis and Lehman 1929.  
 ..... Mean maxillary widths  
 ----- Mean mandibular widths  
 - - - - - Maxillary widths of the individual  
 \_\_\_\_\_ Mandibular widths of the individual.

Between the second molars.

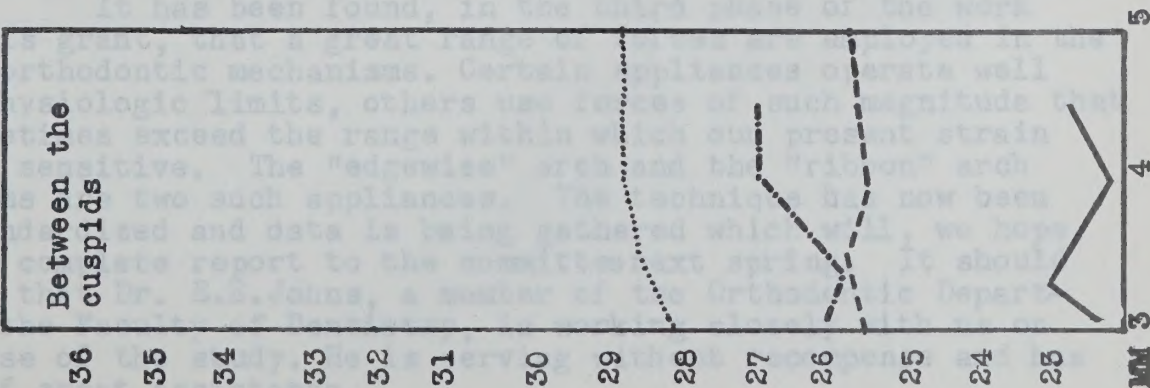


Between the first molars.



Between the cuspids

SAMPLE b2



Robert E. Moyers  
 Signature

"Q-17"





APPENDIX "R"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Measurement of Physiologic Forces in the Mouth

Grantee: Robert E. Moyers

Project: DR 26

As stated in the report presented at the meeting of 2-3 March, 1950, this project has broken down into three separate studies, viz., (a) the measurement of the strength of some of the sucking habits said to be detrimental to the normal growth of the dentition, (b) the measurement of the extent of use of the palatal and pharyngeal muscles in operated and non-operated cleft palate individuals, and (c) the measurement of the forces inherent in the standard orthodontic appliances. They will be discussed separately.

A hydrostatic system making use of a physiological pressure transducer is being used to study the sucking habits. It may be stated that the pressures induced by thumb and finger sucking have been found, in most subjects, to be well above the amounts necessary for alterations in osseous tissue. There is greater variation in the pressures associated with lip and cheek sucking and less often does it appear likely that they may be indicted as being etiologic to dento-facial deformity. Most surprising to us has been the extreme forces sometimes exerted by the tongue. At the present time data is still being gathered as suitable subjects can be found. It is hoped that a complete report, including a statistical analysis of the clinical implications, will be ready for presentation at the spring meeting of the Committee.

The work in regard to the cleft palate muscle actions has gone, thus far, less successfully. Some progress has been made, however, and the muscles are being studied electromyographically as well. The arrival in Toronto of Dr. Ernest Hixon, who is doing research under a grant from the Crippled Children's Grant, has proven helpful since he is an authority in the field of cleft palate care and therapy.

It has been found, in the third phase of the work under this grant, that a great range of forces are employed in the various orthodontic mechanisms. Certain appliances operate well within physiologic limits, others use forces of such magnitude that they sometimes exceed the range within which our present strain gauge is sensitive. The "edgewise" arch and the "ribbon" arch mechanisms are two such appliances. The technique has now been well standardized and data is being gathered which will, we hope, permit a complete report to the committee next spring. It should be noted that Dr. E.E. Johns, a member of the Orthodontic Department of the Faculty of Dentistry, is working closely with us on this phase of the study. He is serving without recompense and has proven of great assistance.

Robert E. Moyers

Signature







APPENDIX "S"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: Development of a method for determining fluorine  
by means of a photo-electric colorimeter.

Grantee: Dr. O. J. Walker

Project: DR 28                      Amount of Grant: \$1,000.

SUMMARY OF WORK

In May 1950, Mr. J. H. Martin presented his Thesis to the Committee of Graduate Studies of the University of Alberta entitled "The Use of the Photoelectric Colorimeter for Determining Small Amounts of Fluorides and Phosphates", in partial fulfillment of the requirements for the Master's Degree. His important conclusions were these:

(1) A satisfactory photoelectric method involving the sodium alizarin sulfonate - zirconyl chloride reaction has been developed.

(2) In this method sulfates up to 150 p.p.m. do not interfere. With greater amounts these can be corrected for by using a suitable correction table.

(3) Phosphates do not interfere if present in quantities of 2 p.p.m. or less. Up to 20 p.p.m. the interference is slight but somewhat irregular.

(4) A photoelectric method for phosphates has been developed which is satisfactory in the presence of fluorides.

Unfortunately it was not possible to obtain a research assistant for the summer months on this project, so very little work has been done since May on the project. It is hoped that some progress will be made during the winter session.

Date: October 10, 1950.

"O. J. Walker"  
Signature





# APPENDIX "T"

## ASSOCIATE COMMITTEE ON DENTAL RESEARCH

### PAPERS PUBLISHED

| <u>Committee<br/>Paper No.</u> | <u>Title and Author</u>                                                                                                                                     | <u>Reference</u>                                                                                                          |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| 1                              | New Aspects of Periodontal Research,<br>by H. K. BOX                                                                                                        | Presented at National Convention of Canadian Dentists, Toronto, 29 May 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb. 1947). |
| 2                              | Concerning the Pathogenesis of Periodontal Disease,<br>by H. K. BOX                                                                                         | J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg., A.C.D.R., 2 Mar. 1948).                                          |
| 3                              | The Effect of Various Inorganic Salts on the Solubility of Calcium Phosphate, Tooth Enamel and Whole Teeth in Lactic Acid,<br>by J. J. RAE and C.T. CLEGG   | J. Dent. Res. 27: 52-53. 1948.                                                                                            |
| 4                              | Changes in the Calcium and Phosphate Concentrations of Saliva and Inorganic Salt Solutions on Shaking with Calcium Phosphate,<br>by J.J. RAE and C.T. CLEGG | J. Dent. Res. 27: 54-57. 1948.                                                                                            |
| 5                              | An Improved Method for Processing Acrylic Dentures,<br>by D.R. CHRISTIE                                                                                     | J. Can. Dent. Assoc., June, 1948. 15 pp. (App. "R", 8th Mtg., A.C.D.R., 26 Oct., 1948).                                   |
| 6                              | The Therapeutic Properties of Periodontal Cement Packs,<br>by W.J. LINGHORNE and D.C. O'CONNELL                                                             | J. Can. Dent. Assoc., April, 1949. 7 pp.                                                                                  |
| 7                              | Phosphatase in Saliva,<br>by J.T. DENTAY and J.J. RAE                                                                                                       | J. Dent. Res., Feb. 1949. 4 pp.                                                                                           |
| 8                              | The Treatment of Acute Oral Vincent's Infection,<br>by W.J. LINGHORNE and D.F. STEDMAN                                                                      | J. Can. Dent. Assoc., July, 1949. 6 pp. (N.R.C. No. 1699).                                                                |





| <u>Committee<br/>Paper No.</u> | <u>Title and Author</u>                                                                                                               | <u>Reference</u>                                      |
|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| 9                              | A Comparison of Nine Local<br>Anaesthetics,<br>by H.S. HAMILTON,<br>B.A. WESTFALL and<br>J.K.W. FERGUSON                              | J. Pharmacol.<br>Expt. Therap.<br>94: 299-307.1948.   |
| 10                             | New Methods of Repairing<br>Acrylic Dentures Without<br>Distortion,<br>by D.R. CHRISTIE                                               | J. Can. Dent.<br>Assoc.,<br>Nov. 1949. 11 pp.         |
| 11                             | The Corrosion of Cobalt Chromium<br>Alloys in Commercial Cleaning<br>Agents, by D.E.R. COGGLES,<br>O.J. WALKER and<br>H.R. MACLEAN    | J. Can. Dent. Assoc.,<br>16: 75-82. 1950.             |
| 12                             | The Relation between Buffering<br>Capacity, Viscosity, and Lacto-<br>bacillus Count of Saliva, by<br>J.J. RAE and<br>C.T. CLEGG       | J. Dent. Res.,<br>28: 589-593. 1949.                  |
| 13                             | Mineralization of the Growing<br>Tooth as Shown by Radio-<br>Phosphorus Autographs (17692),<br>by L.F. BELANGER and<br>C.P. LEBLOND   | Proc. Soc. Expt. Biol.<br>Med., 73: 390-391.<br>1950. |
| 14                             | Radio-autographic Visualization<br>of Bone Formation in the Rat,<br>by C.P. LEBLOND, G.W. WILKINSON,<br>L.F. BELANGER and J. ROBICHON | The Am. J. of Anat.,<br>86: 2, March 1950.            |
| 15                             | Fluoride Studies Related to the<br>Human Diet, by MARY P. HAM and<br>M. DOREEN SMITH                                                  | Can. J. Res.,<br>F, 28: 227-233.<br>1950.             |

S. K. M. Williams  
Signature

The Secretary of the Committee has a small stock  
of most of the papers listed and will supply copies  
to members of the Committee on request.

November, 1950.





APPENDIX "U"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

October, 1950

Subject: The effects of hard and soft diets on the supporting structures of the teeth.

Grantee: Dr. C.H.M. Williams

Project: DR 24                      Amount of Grant: \$1735.

SUMMARY OF WORK

Since the last report, a group of animals which were placed on an experimental basis for a full calendar year have been sacrificed. The material has been prepared for macroscopic and microscopic examination and a report on the findings is in preparation. The findings on this group will be compared with the observations made on the animals which remained only four months on the experimental program.

Observations have been made on adult animals which had been raised to maturity on hard foods. One-half of the group was then fed the same food in a soft pappy form. The experimental period was four months. The mandibles of the hard diet group were greater in volume and weight than those of the soft diet group. Whether this indicates a continuous increase in the size of the mandibles of the animals on the hard diet, or an atrophy of the mandibles of the animals on the soft diet, is being studied by comparison with other control groups. The gingival tissues of the animals on the hard diet were superior to those of the animals on the soft diet, as was previously reported for the first experimental group.

C. H. M. Williams

Signature





## APPENDIX "V"

### DENTAL RESEARCH IN AUSTRALIA AND NEW ZEALAND

by

Roy G. Ellis, Chairman  
Associate Committee on Dental Research

The over-all picture of dental research in these two countries is encouraging. While the amount of research being undertaken in the dental schools in a general way is quite limited, mainly because of accommodation, there have been two interesting developments in Australia and similarly two in New Zealand.

#### Australia

The Commonwealth Bureau of Dental Standards: Some years ago, the Department of Dental Materials in the Australian College of Dentistry of the University of Melbourne, became active under the leadership of a dental graduate, Mr. H. K. Worner. This graduate continued his studies and research and obtained the D.D.Sc. in this field. The research laboratory of the department of Dental Materials expanded greatly and in January, 1947, after some seven or eight years of increasing activity, it was reconstituted as the Commonwealth Bureau of Dental Standards.

The request for the establishment of this Bureau under the Director-General of Health of the Commonwealth Government, was made by Professor A.B.P. Amies in 1944. In 1945, the Australian Dental Association was asked to support this suggestion and the President of the A.D.A. at that time, in furtherance of a motion passed at the annual meeting of the Association, stressed to the Director-General of Health, the necessity for the establishment of a Bureau of Dental Standards. Thus, when the Commonwealth Bureau of Dental Standards was inaugurated in January 1947, it had the financial support of the Commonwealth Government through a grant from the Department of Health, amounting to £ 4,000 per annum, and the moral support of the Australian Dental Association which established the only regular standing Committee known as the "Standards Committee" of the A.D.A.

The Bureau has accommodation, through an arrangement with the University of Melbourne, on the campus of the University of Melbourne. At present they occupy six to eight rooms, and during my visit, there was ample evidence of expansion going on through an addition now under construction. It would appear that the location of the Bureau will remain in Melbourne and that it will continue to be closely affiliated with the University of Melbourne even though it will likely become more and more a Commonwealth Government institution.







The staff of six to eight scientists is directed by Mr. A. R. Docking, M.Sc., who has no dental degree, since Dr. Worner became Head of the University Department of Metallurgy. It is interesting to note that Professor Worner with his initial dental training has assumed this important responsibility in the general field of metallurgy within the University.

From my observations, the Bureau has been excellently equipped with all the latest apparatus for research in the field of dental metallurgy.

A survey of the annual reports published and of the publications which have emanated from the Commonwealth Bureau of Dental Standards indicates that the dental profession will benefit greatly from the standardization of dental materials. Indeed, they have already laid down specifications for some dental materials similar to the specifications set forth by the National Bureau of Standards in Washington.

It would, therefore, seem that future research on dental materials will be centred in Melbourne.

The Institute of Dental Research: The Institute of Dental Research of the United Dental Hospital of Sydney grew out of what was previously the Department of Pathology of the United Dental Hospital. This small department within the Dental Hospital was responsible for providing a limited service in routine clinical pathology. The dental research scholar of the University of Sydney was automatically associated with the Department. The present director of the Institute, Dr. N. E. Goldsworthy, was appointed Director of the Department of Dental Pathology at the United Dental Hospital in July, 1946. At the outset, he believed that it would be expedient to reorganize the Department, so that it would be a separate unit with its own budget and organization, rather than as a Department of Pathology within the Dental Hospital.

The Director, in accordance with this belief, submitted a proposal to the Hospital Commission entitled "Revised Proposals for an Institute of Dental Research." This report, presented in August, 1946, to the Chairman of the Hospital Commission by the President of the Dental Hospital, was supported by the Dean of the Faculty of Dentistry and Dr. Goldsworthy.

During August, 1946, the successful completion of negotiations between the Hospital Commission and the Board of the Dental Hospital resulted in a resolution by the Board of the Hospital as follows: "that there be founded the Institute of Dental Research in accordance with the proposals presented to the Board and to the Hospital Commission." The Commission was notified of this resolution, and late in August replied that it was agreeable to the foundation of the Institute and to its name; further it had agreed to make a grant to the Institute.







This action abolished the Hospital's Department of Pathology, Bacteriology, and Biochemistry. In addition, with the establishment of the Institute, the staff, accommodation, equipment and supplies were transferred from the old to the new Institute. Actually, they remained in the same quarters in the Dental Hospital, as they had occupied previously. Thus, during the first year, the Institute included departments of:

1. Pathological Anatomy and Histopathology
2. Bacteriology and Immunology
3. Biochemistry and Clinical Pathology

While their present accommodation is very limited and might be described as shockingly inadequate, the completion of the present elaborate building program for the Dental Hospital will provide the Institute of Dental Research with the entire seventh floor of the building amounting to forty rooms. An elaborate staff plan has been prepared and at the present time comprises nine full-time people and three part-time.

The Research Institute is financed today, according to my conversations with the Director, through the following sources:

1. A direct grant from the Hospital Commission.  
(Amount unstated).
2. From the Dental Hospital which pays for routine examinations of pathological and biochemical specimens.
3. A small grant from the Dental Board of the State of New South Wales.
4. Such applications for grants-in-aid for specified projects directed by individuals; in this case, the Director of the Institute. These grants-in-aid come from the Medical Research Council.

It will be seen, therefore, that as in the case of the Commonwealth Bureau of Standards, so in the case of the Institute of Dental Research, it had its beginnings in a department within the Dental School of a University.

#### NEW ZEALAND

Dental research in New Zealand is in evidence in two locations; namely, the Dental School at the University of Otago, and the Division of Dental Hygiene of the Department of Health of the New Zealand Government in Wellington. The latter, under the direction of the Director of the Division of Dental Hygiene is closely associated with the training School for New Zealand Dental Nurses in Wellington.





The Dental School: A fairly active program of research is underway in three major fields in the Dental School.

1. A study of the masticatory mechanism which is engaging the attention of at least three departments. The Dean made a special point to emphasize the fact that with New Zealand's high incidence of dental caries and the mortality of teeth in young adults, a great variety of temporo-mandibular disturbances are seen. It is natural, therefore, that they are interested in the problem from a research standpoint.

2. Some studies are progressing on the physico-chemical aspects of dental caries. The research worker in this case is not a dental graduate, but a student proceeding to his Master's degree in chemistry.

3. A project on filling materials and the dental pulp is also included.

The sources of funds for the support of research in the Dental School are as follows:

1. £ 2,000 a year from the New Zealand Medical Research Council.
2. £1,000 (Capital Grant) which is being used for equipment.
3. £13,000 (Capital Grant) the interest of which is being used to establish Research Fellowships.

The very active and enthusiastic young staff at the Dental School have plans for considerable expansion of their dental research activities when their new dental building is completed. Plans for the new building have been approved, the site purchased and it is anticipated that the Government will proceed with construction work in the near future.

The Division of Dental Hygiene: Under the Division of Dental Hygiene, a program of dental research in the field of Public Health Research is underway. Dr. R.E.T. Hewat was appointed Field Research Officer in June, 1947. He has been engaged in a study of regional variations in the incidence of dental caries in New Zealand. In conversations with Dr. Hewat and the Director of the Division of Dental Hygiene, I was informed that their efforts in the research field would be limited to studies of this nature.

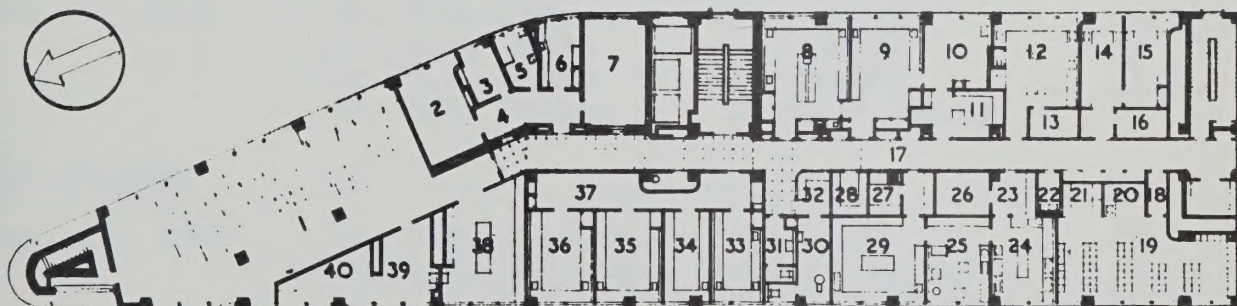
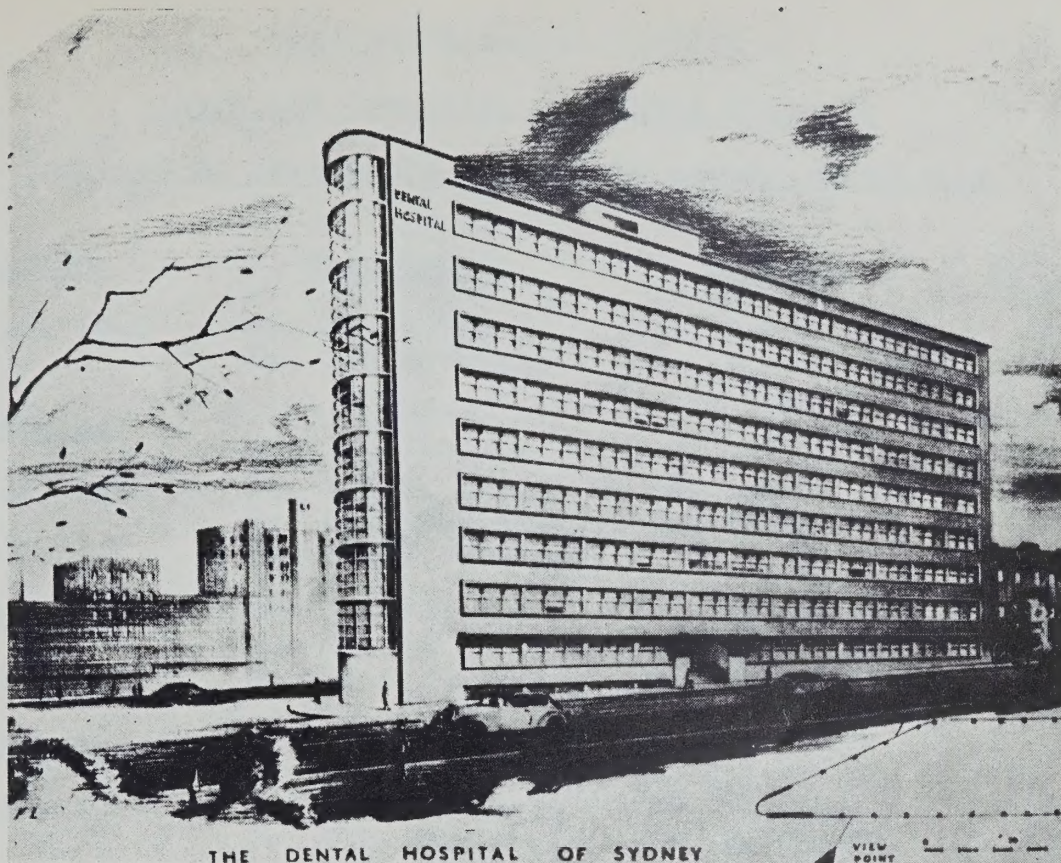
Faculty of Dentistry,  
University of Toronto,  
Toronto, Ont.

6 October, 1950.









SEVENTH FLOOR PLAN

SEVENTH FLOOR LEGEND

- |                            |                        |                       |                         |
|----------------------------|------------------------|-----------------------|-------------------------|
| 1 FAN ROOM                 | 11 CLERK               | 21 CAGE CLEANING      | 31 STAFF LAVATORY       |
| 2 CARETAKER'S QRS. BEDROOM | 12 CHEMICAL LABORATORY | 22 CLEANER'S CUPBOARD | 32 NURSE'S STATION      |
| 3 BEDROOM                  | 13 BALANCE ROOM        | 23 LOBBY              | 33 RESEARCH LABORATORY  |
| 4 ENTRANCE HALL            | 14 SECTION CUTTING     | 24 WASH ROOM          | 34 RESEARCH LABORATORY  |
| 5 BATH ROOM                | 15 MUSEUM PREPARATION  | 25 STERILIZING ROOM   | 35 RESEARCH LABORATORY  |
| 6 KITCHEN                  | 16 STORE               | 26 STORE              | 36 RESEARCH LABORATORY  |
| 7 LIVING ROOM              | 17 CORRIDOR            | 27 CLERK              | 37 CLERKS' OFFICE       |
| 8 ROUTINE LABORATORY       | 18 AIR-LOCK            | 28 CENTRIFUGE ROOM    | 38 CARPENTER'S WORKSHOP |
| 9 DIRECTOR'S LABORATORY    | 19 ANIMAL QUARTERS     | 29 MEDIA PREPARATION  | 39 PAINTERS WORKSHOP    |
| 10 DIRECTOR'S OFFICE       | 20 FODDER ROOM         | 30 EXAMINATION ROOM   | 40 PAINTERS WORKSHOP    |

THE INSTITUTE OF DENTAL RESEARCH.





APPENDIX "W"  
(in the 12th Meeting as revised)

National Research COUNCIL

Associate Committee on Dental Research

Grantees or Assistants invited to report personally

| <u>3rd Meeting</u>  | <u>Reporting</u>    | <u>Grantee</u>     | <u>Project No.</u> |
|---------------------|---------------------|--------------------|--------------------|
| Feb. 18, 1946       | Dr. J. J. Rae       | Dr. Rae            | DR 1               |
|                     | Dr. G.H.W. Lucas    | Dr. Lucas          | DR 2               |
| <u>4th Meeting</u>  |                     |                    |                    |
| Oct. 22, 1946       | Dr. G.H.W. Lucas    | Dr. Lucas          | DR 2               |
|                     | Mr. D.R. Christie)  | Dr. Westman        | DR 5               |
|                     | Dr. A.E.R. Westman) |                    |                    |
| <u>5th Meeting</u>  |                     |                    |                    |
| Feb. 25, 1947       | Dr. J. S. Bagnall   | Dr. Bagnall        | DR 7               |
| <u>6th Meeting</u>  |                     |                    |                    |
| Oct. 21, 1947       | Mr. D.R. Christie   | Dr. Westman        | DR 5               |
|                     | Dr. G.M. MacDonald  | Dr. MacDonald      | DR 10              |
|                     | Dr. C.H. Moses      | Dr. Godfrey        | DR 11              |
|                     | Mr. D.R. Christie   | Dr. Westman        | DR 12              |
| <u>7th Meeting</u>  |                     |                    |                    |
| Mar. 2, 1948        | Dr. J.S. Bagnall    | Dr. Bagnall        | DR 7               |
|                     | Dr. M.D. Smith      | Dr. Smith          | DR 13              |
|                     | Dr. M.D. Smith      | Dr. Smith          | DR 14              |
|                     | Dr. N.F. Walker     | Dr. N.F. Walker    | DR 15              |
| <u>8th Meeting</u>  |                     |                    |                    |
| Oct. 26, 1948       | Dr. G.H.W. Lucas    | Dr. Lucas          | DR 2               |
|                     | Dr. G.M. MacDonald  | Dr. MacDonald      | DR 10              |
|                     | Dr. C.H. Moses      | Dr. R.J. Godfrey   | DR 11              |
| <u>9th Meeting</u>  |                     |                    |                    |
| Mar. 3-4, 1949      | Dr. O.J. Walker     | Walker and MacLean | DR 9               |
|                     | Dr. M.D. Smith      | Dr. Smith          | DR 13              |
|                     | Mr. M.D. Smith      | Dr. Smith          | DR 14              |
|                     | Dr. R.A. Hugill     | Dr. H.K. Box       | DR 18              |
|                     | Dr. J. Thébaud      | Dr. Thébaud        | DR 20              |
| <u>10th Meeting</u> |                     |                    |                    |
| Oct. 17-18, 1949    | Dr. G.H.W. Lucas    | Dr. Lucas          | DR 2               |
|                     | Dr. J.S. Bagnall    | Dr. Bagnall        | DR 7               |
|                     | Mr. D.R. Christie   | Dr. Westman        | DR 12              |
|                     | Mr. D.R. Christie   | Dr. Westman        | DR 21              |
|                     | Dr. W.J. Linghorne  | Dr. Best           | DR 22              |
|                     | Dr. L.F. Bélanger   | Dr. Leblond        | DR 23              |





| <u>Project</u>      | <u>Reporting</u>          | <u>Grantee</u>                  | <u>Project No.</u> |
|---------------------|---------------------------|---------------------------------|--------------------|
| <u>11th Meeting</u> | 1 (member of Committee)   | 1                               | 2                  |
| Mar. 2-3, 1950      | Mr. C.T. Clegg            | Dr. J.J. Rae                    | DR 1               |
|                     | Dr. M.D. Smith            | Dr. Smith                       | DR 13              |
| DR 3                | Dr. N.F. Walker           | Dr. Walker                      | DR 15              |
|                     | Dr. R.A. Hugill           | Dr. Box                         | DR 18              |
| DR 4                | Dr. D.G. Watt             | Dr. C.H.M. Williams             | DR 24              |
|                     | Dr. A.G. Nutlay           | Drs. J.W. Abbiss and Nutlay     | DR 27              |
| DR 5                |                           |                                 |                    |
|                     | Dr. M.D. Smith            | Dr. Smith                       | DR 29              |
| DR 6                | Dr. N.F. Walker           | Dr. Walker                      | DR 30              |
| DR 7                |                           |                                 |                    |
| <u>12th Meeting</u> |                           |                                 |                    |
| Oct. 16-17, 1950    | Dr. N.F. Walker           | Dr. Walker                      | DR 15              |
| DR 8                | Dr. W.J. Linghorne        | Dr. Best                        | DR 22              |
| DR 9                | Dr. W.L. Liu              | Dr. Walker                      | DR 30              |
| DR 10               | Mr. G.J. Millar           | Dr. L.B. Jacques and Mr. Millar | DR 31              |
| DR 11               | Dr. J.B. Macdonald        | Dr. Macdonald                   | DR 35              |
| DR 12               |                           |                                 |                    |
| <u>13th Meeting</u> |                           |                                 |                    |
| Mar 8-9             |                           |                                 |                    |
| DR 15               | D. R. Christie            | Nestman                         | DR 12 x 25         |
| DR 16               | A. D'Zio                  | D'Zio                           | D.R. 34            |
| DR 17               | & Kossow                  |                                 |                    |
| DR 18               | R. L. Mayers et al Mayers |                                 | D.R. 26            |
| DR 19               | <del>D. M. D. Smith</del> | <del>M. D. Smith</del>          | <del>D.R. 13</del> |
| DR 20               |                           |                                 |                    |
| DR 21               |                           |                                 |                    |
| DR 22               |                           |                                 |                    |
| DR 23               |                           |                                 |                    |
| DR 24               |                           |                                 |                    |
| DR 25               |                           |                                 |                    |





| <u>Project</u> | <u>Grantee</u>          | <u>Assistant</u> | <u>Total</u> |
|----------------|-------------------------|------------------|--------------|
| DR 1           | 1 (member of Committee) | 1                | 2            |
| DR 2           | 4                       |                  | 4            |
| DR 3           | 1 (member of Committee) |                  | 1            |
| DR 4           | 1 (member of Committee) |                  | 1            |
| DR 5           | 1                       | 2                | 3            |
| DR 6           | 1 (member of Committee) | -                | 1            |
| DR 7           | 3                       | -                | 3            |
| DR 8           | 0                       | -                | 0            |
| DR 9           | 1                       | -                | 1            |
| DR 10          | 2                       | -                | 2            |
| DR 11          | -                       | 2                | 2            |
| DR 12          | -                       | 2                | 2            |
| DR 13          | 3                       | -                | 3            |
| DR 14          | 2                       | -                | 2            |
| DR 15          | 3                       | -                | 3            |
| DR 16          | 1 (member of Committee) |                  | 1            |
| DR 17          | 0                       | -                | 0            |
| DR 18          | -                       | 2                | 2            |
| DR 19          | 0                       | -                | 0            |
| DR 20          | 1                       | -                | 1            |
| DR 21          | -                       | 1                | 1            |
| DR 22          | -                       | 2                | 2            |
| DR 23          | -                       | 1                | 1            |
| DR 24          | -                       | 1                | 1            |
| DR 25          | 1                       | -                | 1            |





reported by:

| <u>Project</u> | <u>Grantee</u> | <u>Assistant</u> | <u>Total</u> |
|----------------|----------------|------------------|--------------|
| DR 26          | 0              | 0                | 0            |
| * DR 27        | 1              | -                | 1            |
| DR 29          | 1              | -                | 1            |
| DR 30          | 1              | 1                | 2            |
| DR 31          | 1              | -                | 1            |
| DR 35          | 1              | -                | 1            |

Representing the Dental Profession

3. Dr. H. E. Box,  
Professor of Periodontology,  
University of Toronto,  
Toronto, Ont. 31 March, 1952
- \* 4. Dr. E. Charron,  
Dean of the Faculty of Dental Surgery,  
University of Montreal,  
Montreal, Que. 31 March, 1951
5. Dr. W. H. Garvin,  
Editor-in-Chief,  
Journal of the Canadian Dental Association,  
314 Somerset Building,  
Winnipeg, Man. 31 March, 1952
6. Dr. H. R. MacLean,  
Lecturer in Operative Dentistry,  
Faculty of Dentistry,  
University of Alberta,  
Edmonton, Alta. 31 March, 1952
7. Dr. R. H. McDougall,  
1505 Hampshire Road,  
Victoria, B.C. 31 March, 1952

Representing the Royal Canadian Dental Corps

- \* 8. Col. E. M. Macdonough (ex officio),  
Director General of Dental Services,  
Royal Canadian Dental Corps,  
Department of National Defence,  
Ottawa, Ont.





2 -

Representing the Division of Dental Health  
Department of National Health and Welfare,  
Ottawa, Ont.

Members of the Associate Committee on Dental  
Research and its Executive, with their terms  
of appointment

---

Term to Expire

\* 1. Dr. R. G. Ellis, Chairman  
Dean of the Faculty of Dentistry,  
University of Toronto,  
Toronto, Ont. 31 March, 1951

2. Dr. C. J. Mackenzie (ex officio),  
President  
National Research Council,  
Ottawa, Ont. -

Representing the Dental Profession

3. Dr. H. K. Box,  
Professor of Periodontology,  
University of Toronto,  
Toronto, Ont. 31 March, 1952

\* 4. Dr. E. Charron,  
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5. Dr. M. H. Garvin,  
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\* 8. Col. E. M. Wansbrough (ex officio),  
Director General of Dental Services,  
Royal Canadian Dental Corps,  
Department of National Defence,  
Ottawa, Ont. -

\* 18. Mr. S. J. Cook, Secretary,  
Public Relations Branch,  
National Research Council,  
Ottawa, Ont.





Representing the Division of Dental Health  
Department of National Health and Welfare

9. Dr. H. K. Brown, (ex officio),  
Chief, Dental Health Division,  
Department of National Health and Welfare,  
Ottawa, Ont.

Representing the Basic and Medical Sciences

10. Dr. C. H. Best,  
Director, Banting and Best Department of  
Medical Research,  
University of Toronto,  
Toronto, Ont. 31 March, 1953
11. Dr. J. B. Collip,  
Dean of the Faculty of Medicine,  
University of Western Ontario,  
London, Ont. 31 March, 1952
12. Dr. O. W. Ellis,  
Department of Metallurgy,  
Ontario Research Foundation,  
Toronto, Ont. 31 March, 1951
13. Dr. J.K.W. Ferguson,  
Head of the Department of Pharmacy  
and Pharmacology,  
University of Toronto,  
Toronto, Ont. 31 March, 1951
14. Dr. A. W. Ham,  
Professor of Anatomy,  
Faculty of Medicine,  
University of Toronto,  
Toronto, Ont. 31 March, 1952
15. Dr. J. J. Rae,  
Department of Chemistry,  
University of Toronto,  
Toronto, Ont. 31 March, 1953
16. Dr. C.B. Stewart,  
Faculty of Medicine,  
Dalhousie University,  
Halifax, N.S. (J.B. MacDonald) 31 March, 1951
- \* 17. Dr. D.L. Thomson,  
Chairman of the Department of Biochemistry,  
McGill University,  
Montreal, Que. 31 March, 1953
- \* 18. Mr. S. J. Cook, Secretary,  
Public Relations Branch,  
National Research Council,  
Ottawa, Ont. -

\* MEMBERS OF THE EXECUTIVE





## INITIAL DISTRIBUTION

### (i) To Members of the

#### Associate Committee on Dental Research

- |                                       |                                     |
|---------------------------------------|-------------------------------------|
| 1. Dr. R. G. Ellis, <u>Chairman</u> ✓ | 10. Dr. A. W. Ham ✓                 |
| 2. Dr. C. H. Best 0                   | 11. Dr. C.J. Mackenzie(ex officio)  |
| * 3. Dr. H. K. Box 0                  | 12. Dr. H.R. MacLean ✓              |
| 4. Dr. H. K. Brown(ex officio)✓       | 13. Dr. R.H. McDougall 0            |
| 5. Dr. E. Charron ✓                   | 14. Dr. J.J. Rae ✓                  |
| 6. Dr. J. B. Collip 0                 | * 15. Dr. C.B. Stewart ✓            |
| 7. Dr. O. W. Ellis ✓                  | 16. Dr. D.L. Thomson ✓              |
| 8. Dr. J.K.W. Ferguson 0              | 17. Col. E.M.Wansbrough(ex officio) |
| 9. Dr. M.H. Garvin ✓                  | 18. Mr. S.J. Cook, Secretary ✓      |

### (ii) To Other Persons:

19-21 Deans of Dental Schools (who are not members of the Committee)

19. Alberta - Dr. W.S. Hamilton

20. Dalhousie - Dr. J.S. Bagnall

21. McGill - Dr. D. Prescott Mowry

22. Master Copy (General Secretary)

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
OF THE  
TENTH MEETING  
OF THE  
EXECUTIVE  
ASSOCIATE COMMITTEE  
ON DENTAL RESEARCH

OTTAWA

8 MARCH, 1951







NATIONAL RESEARCH COUNCIL

Project No. Name of Applicant Proceedings

DR 13 Dr. M. D. ... Tenth Meeting

DR 22 Dr. C. H. ... of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Council Chamber, 4 P.M., 8 March 1951

1. Attendance

Dr. R. G. Ellis, Chairman  
Dr. C. J. Mackenzie, President  
Dr. E. Charron  
Dr. A. W. Ham  
Dr. D. L. Thomson  
Col. E. M. Wansbrough

DR Mr. S. J. Cook, Secretary

2. Consideration of Applications for Renewals of Grants

Applications for renewals of grants for 1951-52 were considered and action was taken as indicated hereunder:

| <u>Project No.</u> | <u>Name of Applicant</u>                         | <u>Amount of Grant</u> |
|--------------------|--------------------------------------------------|------------------------|
| DR 1               | Dr. J.J. Rae<br><u>Action:</u> Recommended       | \$<br>960              |
| DR 12 and 25       | Dr. A.E.R. Westman<br><u>Action:</u> Recommended | 4000                   |

It was pointed out that something might be gained by having Mr. D. R. Christie visit the National Bureau of Standards where work is proceeding on methacrylate fillings, in order that activities in this field may be properly correlated.

DR 33 It was MOVED by Col. Wansbrough and SECONDED by Dr. Charron,

That the Executive recommends that arrangements be made for Mr. D. R. Christie to be sent to the National Bureau of Standards, Washington, D.C., U.S.A., for a week to study work in progress there on methacrylate fillings in order that he may correlate his own program with that work.  
CARRIED.





| <u>Project No.</u> | <u>Name of Applicant</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <u>Amount of Grant</u> |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| DR 13              | Dr. M. Doreen Smith<br><u>Action:</u> Recommended                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | \$<br>1,600            |
| DR 22              | Dr. C. H. Best \$5000<br><u>Action:</u> There was considerable discussion on this application and it was agreed that the work on repair and attachment being done by Dr. Linghorne under this grant should be supported but the Executive decided to obtain further opinions regarding the bacteriological side being conducted by Dr. O'Connell. It was finally agreed to vote \$2300 under this application and to provide a supplementary unstated amount to be determined after consultation by the Chairman, the total of the grant not to exceed \$5,000. | 2,300                  |
| DR 23              | Dr. C. P. Leblond<br><u>Action:</u> The application was for \$2400 and it was AGREED to suggest to Dr. Leblond that \$200 additional would be awarded him under his 1950-51 grant for the purchase of items that might be bought before 31 March 1951.                                                                                                                                                                                                                                                                                                          | 2,200                  |
| DR 28              | Dr. O. J. Walker<br><u>Action:</u> Recommended                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1,000                  |
| DR 31              | Dr. L. B. Jaques and<br>Mr. G. J. Millar \$2,000<br><u>Action:</u> The Executive decided to consult Dr. J.K.W. Ferguson in regard to this application but not to make the grant unless he very strongly favours it.                                                                                                                                                                                                                                                                                                                                             | -                      |
| DR 33              | Dr. H. A. Hunter<br><u>Action:</u> Recommended<br>Dr. Hunter requests that the name of this project be changed to "The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure".                                                                                                                                                                                                                                                                                                                       | 1,600                  |





| <u>Project No.</u> | <u>Name of Applicant</u>                                                                                                                                                                                                                              | <u>Amount of Grant</u> |
|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| DR 34              | Dr. J. P. Lussier (formerly in the name of Dr. A.D'Iorio)<br><u>Action:</u> It was AGREED to recommend that \$850 additional be awarded to Dr. Lussier under his 1950-51 grant for the purchase of equipment that can be bought before 31 March 1951. | \$<br>350              |
| DR 35              | Dr. J. B. Macdonald<br><u>Action:</u> Recommended<br>It was AGREED also to recommend an additional grant of \$200 under this project from 1950-51 funds for the purchase of equipment that can be bought before 31 March 1951.                        | 2,600                  |

Total amounts recommended for award under renewal applications:

- (i) From 1950-51 - \$1250
- (ii) From 1951-52 funds - \$16,610

### 3. New Applications

| <u>Grant No.</u> | <u>Name of Applicant</u>                                                                                                                                      | <u>Short title</u>                                                             | <u>Amount</u> |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------|
| DR 36            | Gordon Nikiforuk<br>Assoc.Prof.of Pedodontics<br>Dept. of Dental Research<br>University of Toronto<br>Toronto, Ont.<br><br><u>Action:</u> Recommended         | Demineralization of hard tissues as bone and teeth by organic chelating agents | \$<br>2,400   |
| DR 37            | R. E. Moyers<br>Prof. and Head,<br>Dept.of Orthodontics,<br>Faculty of Dentistry,<br>University of Toronto<br>Toronto, Ont.<br><br><u>Action:</u> Recommended | Further studies in the electromyography of the head, face and neck             | 960           |
| DR 38            | Kenneth Jack Paynter,<br>Faculty of Dentistry,<br>University of Toronto,<br>Toronto, Ont.<br><br><u>Action:</u> Recommended                                   | The effect of Thiouracil on the development of molar teeth of rats             | 1,850         |





- (i) F. Hugh Lawford  
Technical Director,  
Consumers' Research Laboratories  
Limited,  
Toronto, Ont.

The effect of carbohydrate  
on dental caries.

Action: THE CHAIRMAN asked President Mackenzie to comment on this application.

THE PRESIDENT said that there were three points to be considered (i) the problem, (ii) is the applicant competent to carry out the work proposed, (iii) what is the relationship between the National Research Council and an industrial organization in respect of grants in aid?. Commenting on these points, he said that the National Research Council prefers to deal with universities regarding grants in aid because the secondary controls i.e. the professors and the tradition in respect of the handling of grants, have been well developed between the Council and universities. In making a grant to an industrial organization there were not the same safeguards in the management of the grant. In its relations with industry the Council preferred to enter into a contract for an ad hoc job because in this way the details of management were well defined and accepted by both parties. He said that in his opinion the importance of the problem was the principal item to be considered and he thought the Committee should obtain expert advice in this matter.

DR. HAM said that if the problem presented were considered suitable by the Committee perhaps the situation could be dealt with by giving the assistant concerned a fellowship.

PRES. MACKENZIE pointed out that it was contrary to Council policy to pay the salary of a person who is a permanent member of an industrial or university organization.

On consideration it was decided to obtain an opinion from Dr. J. J. Rae on this matter before reaching a decision.

- (ii) Dr. H. A. Hunter,  
Assoc. Prof. of Dentistry,  
Faculty of Dentistry,  
University of Toronto,  
Toronto, Ont.

A histologic study of the teeth and jaws of rats placed on a vitamin B complex deficient diet.

Action: On consideration of this application it was AGREED that insufficient information had been provided by the applicant and it was agreed to ask for more information and to leave the decision as to whether the grant should be made to the Executive.

The total amount recommended for award under new applications was \$5210.





#### 4. Fellowships

Two applications for fellowships were received and both were recommended for award as follows:

(i) Dr. Gerald Shklar

Oral Pathology and Bacteriology

Place of Research: Tufts College Dental School, under Dr. Irving Glickman.

Amount of Award Recommended: \$1500.

(ii) Dr. Willa L. Liu (Chou)

A Study of Migration of Tooth Germs before and during Mixed Dentition.

This work will be carried on in the Department of Orthodontics, Faculty of Dentistry, University of Toronto.

Amount of Award Recommended: \$2200.

#### 5. Amount of Awards 1951-52

The total amount of awards recommended for 1951-52 is as follows:

|                                          |          |
|------------------------------------------|----------|
| (i) Renewals of grant applications ..... | \$16,610 |
| (ii) New applications .....              | 5,210    |
|                                          | <hr/>    |
|                                          | 21,820   |
| (iii) Fellowships .....                  | 3,700    |
|                                          | <hr/>    |
| Total .....                              | \$25,520 |

#### 6. Membership of Committee

THE CHAIRMAN reported that four members of the Committee would complete their second term of office on 31 March 1951, and it was the general rule in committee memberships that members having served two terms should drop out for at least one year before being eligible for new appointments.

But in the case of Dr. J.B. Macdonald, who had recently been appointed a member of the Committee to finish out the unexpired portion of Dr. C.B. Stewart's term, he would be eligible for an initial appointment of three years if the Executive so recommended it.





PRES. MACKENZIE said that while the rule of non-renewal of a membership following a two-term tenure was a sound procedure in general, he thought that the Committee should be careful not to lose the continuity of dental direction of the Associate Committee on Dental Research. He therefore recommended that consideration be given to the nomination of Dr. R.G. Ellis and Dr. E. Charron for a further term of three years. He pointed out that the Associate Committee on Dental Research is a comparatively new organization, and that it is functioning extremely well under its present direction. He saw no objection to applying the rules of membership in the case of members of the Committee who represent the basic sciences, but he felt strongly that the dental direction of this Committee should be retained. He pointed out that both Dr. R.G. Ellis and Dr. E. Charron represent important sections of the dental profession and he said he was sure that the National Research Council would be very pleased to receive a recommendation that both of these members should be asked to serve at least one further term.

Considerable discussion followed in which several members of the Executive expressed their concurrence in the views put forward by President Mackenzie. Several suggestions were offered, and it was finally agreed to select a pathologist and a biophysicist for nomination to the other two vacancies on the Committee.

The following recommendations were subsequently agreed upon:

- (i) That Dr. R.G. Ellis and Dr. E. Charron be nominated for a further term of three years each from 1 April 1951;
- (ii) That the following be nominated for a term of three years, from 1 April 1951:

DR. A. C. BURTON, Professor and Head of the Department of Biophysics, University of Western Ontario, London, Ont.

DR. J.D. HAMILTON, Professor of Pathology,  
Queen's University, Kingston, Ont.

DR. J.B. MACDONALD, Assistant Professor of Bacteriology,  
University of Toronto, Toronto, Ont.

- (iii) That the Director of Dental Services, Department of Veterans' Affairs, Ottawa, be made a member, ex officio, in order to complete representation in the membership of the Committee from the three large dental groups in the Government services.

The Meeting adjourned at 6:15 P.M.





Members of the Associate Committee on Dental Research  
and its Executive, with their terms of appointment

|                                                                                                                                 | <u>Term to Expire</u> |
|---------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| * 1. Dr. R. G. Ellis, Chairman,<br>Dean of the Faculty of Dentistry,<br>University of Toronto,<br>Toronto, Ont.                 | 31 March, 1951        |
| 2. Dr. C. J. Mackenzie, (ex officio)<br>President,<br>National Research Council,<br>Ottawa, Ont.                                | -                     |
| <u>Representing the Dental Profession</u>                                                                                       |                       |
| 3. Dr. J. S. Bagnall,<br>Dean, Faculty of Dentistry,<br>Dalhousie University,<br>Halifax, N.S.                                  | 31 March, 1952        |
| * 4. Dr. E. Charron,<br>Dean of the Faculty of Dental Surgery,<br>University of Montreal,<br>Montreal, Que.                     | 31 March, 1951        |
| 5. Dr. M. H. Garvin,<br>Editor-in-Chief,<br>Journal of the Canadian Dental Assoc.,<br>314 Somerset Bldg.,<br>Winnipeg, Man.     | 31 March, 1953        |
| 6. Dr. H. R. MacLean,<br>Lecturer in Operative Dentistry,<br>Faculty of Dentistry,<br>University of Alberta,<br>Edmonton, Alta. | 31 March, 1953        |
| 7. Dr. R. H. McDougall,<br>1505 Hampshire Road,<br>Victoria, B.C.                                                               | 31 March, 1952        |

Representing the Royal Canadian Dental Corps

|                                                                                                                                                                |   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| * 8. Col. E. M. Wansbrough, (ex officio)<br>Director General of Dental Services,<br>Royal Canadian Dental Corps,<br>Dept. of National Defence,<br>Ottawa, Ont. | - |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|---|





Representing the Division of Dental Health,  
Department of National Health and Welfare

9. Dr. H. K. Brown (ex officio),  
Chief, Dental Health Division  
Dept. of National Health and Welfare,  
Ottawa, Ont.

Representing the Basic and Medical Sciences

10. Dr. C. H. Best, 31 March, 1953  
Director, Banting and Best Dept. of  
Medical Research,  
University of Toronto,  
Toronto, Ont.
11. Dr. J. B. Collip, 31 March, 1952  
Dean of the Faculty of Medicine,  
University of Western Ontario,  
London, Ont.
12. Dr. O. W. Ellis, 31 March, 1951  
Dept. of Metallurgy,  
Ontario Research Foundation,  
Toronto, Ont.
13. Dr. J.K.W. Ferguson, 31 March, 1951  
Head of the Dept. of Pharmacy  
and Pharmacology,  
University of Toronto,  
Toronto, Ont.
14. Dr. A. W. Ham, 31 March, 1952  
Prof. of Anatomy,  
Faculty of Medicine,  
University of Toronto,  
Toronto, Ont.
15. Dr. J. B. Macdonald, 31 March, 1951  
Assistant Prof. of Bacteriology,  
Faculty of Dentistry,  
University of Toronto,  
Toronto, Ont.
16. Dr. J. J. Rae, 31 March, 1953  
Dept. of Chemistry,  
University of Toronto,  
Toronto, Ont.





\* 17. Dr. D. L. Thomson,  
Chairman of the Department of  
Biochemistry,  
McGill University,  
Montreal, Que.

31 March, 1953

\* 18. Mr. S. J. Cook, Secretary,  
Public Relations Branch,  
National Research Council,  
Ottawa, Ont.

\* Members of the Executive

- |                          |                                      |
|--------------------------|--------------------------------------|
| 1. Dr. J. D. Macdonald   | 10. Dr. A. G. Lee                    |
| 2. Dr. J. D. Macdonald   | 11. Dr. J. D. Macdonald              |
| 3. Dr. J. D. Macdonald   | 12. Dr. C. J. Macdonald              |
| 4. Dr. H. K. Brown       | 13. Dr. H. K. Brown                  |
| 5. Dr. J. D. Macdonald   | 14. Dr. H. K. Brown                  |
| 6. Dr. J. D. Macdonald   | 15. Dr. J. D. Macdonald              |
| 7. Dr. D. W. Ellis       | 16. Dr. D. W. Ellis                  |
| 8. Dr. J. K. W. Ferguson | 17. Col. G. H. Macdonald             |
| 9. Dr. H. K. Garvin      | 18. Mr. S. J. Cook, <u>Secretary</u> |

(11) To Other Persons:

- 19-20. Deans of Dental Schools (who are not members of the Committee)
19. Alberta - Dr. W. H. Hamilton
20. McGill - Dr. D. Prescott Perry
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INITIAL DISTRIBUTION

(i) To Members of the

Associate Committee on Dental Research

- |                                     |                                      |
|-------------------------------------|--------------------------------------|
| 1. Dr. R. G. Ellis, <u>Chairman</u> | 10. Dr. A. W. Ham                    |
| 2. Dr. C. H. Best                   | 11. Dr. J. B. Macdonald              |
| 3. Dr. J. S. Bagnall                | 12. Dr. C. J. Mackenzie              |
| 4. Dr. H. K. Brown                  | 13. Dr. H. R. MacLean                |
| 5. Dr. E. Charron                   | 14. Dr. R. H. McDougall              |
| 6. Dr. J. B. Collip                 | 15. Dr. J. J. Rae                    |
| 7. Dr. O. W. Ellis                  | 16. Dr. D. L. Thomson                |
| 8. Dr. J. K. W. Ferguson            | 17. Col. E. M. Wansbrough            |
| 9. Dr. M. H. Garvin                 | 18. Mr. S. J. Cook, <u>Secretary</u> |

(ii) To Other Persons:

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19. Alberta - Dr. W. S. Hamilton
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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
OF THE  
THIRTEENTH MEETING  
OF THE  
ASSOCIATE COMMITTEE ON  
DENTAL RESEARCH

OTTAWA

8-9 MARCH 1951







## TABLE OF CONTENTS

|                                                 | <u>Page</u> |
|-------------------------------------------------|-------------|
| 1. Attendance .....                             | 1           |
| 2. Chairman's Remarks .....                     | 1           |
| 3. Minutes of the 12th Meeting .....            | 2           |
| 4. Business Arising out of the Minutes ....     | 2           |
| 5. DR 1 - Dr. J. J. Rae .....                   | 5           |
| 6. DR 2 - Dr. G.H.W. Lucas .....                | 5           |
| 7. DR 12 - Dr. A.E.R. Westman .....             | 5           |
| 8. DR 13 - Dr. M. Doreen Smith .....            | 5           |
| 9. DR 15 - Dr. Norma Ford Walker .....          | 6           |
| 10. DR 22 - Dr. C. H. Best .....                | 6           |
| 11. DR 23 - Dr. C. P. Leblond .....             | 7           |
| 12. DR 24 - Dr. C.H.M. Williams .....           | 7           |
| 13. DR 25 - Dr. A.E.R. Westman .....            | 7           |
| 14. DR 26 - Dr. R. E. Moyers .....              | 9           |
| 15. DR 27 - Dr. A. G. Nutlay .....              | 10          |
| 16. DR 28 - Dr. O. J. Walker .....              | 10          |
| 17. DR 29 - Dr. M. Doreen Smith .....           | 11          |
| 18. DR 30 - Dr. Norma Ford Walker .....         | 11          |
| 19. DR 31 - Dr. L.B. Jaques and Mr. G.J. Millar | 11          |
| 20. DR 32 - Dr. A. W. Ham .....                 | 12          |
| 21. DR 33 - Dr. H. A. Hunter .....              | 12          |
| 22. DR 34 - Dr. A. D'Iorio .....                | 12          |
| 23. DR 35 - Dr. J. B. Macdonald .....           | 13          |
| 24. Finances .....                              | 13          |
| 25. Air Travel (1951-52) .....                  | 14          |
| 26. New Business .....                          | 14          |
| 27. Visitors at the Next Meeting .....          | 15          |





TABLE OF CONTENTS

|                                       | <u>Page</u> |
|---------------------------------------|-------------|
| 28. Attendance (Second Session) ..... | 15          |
| 29. Demonstration by Dr. Moyers ..... | 16          |
| 30. Executive Meeting .....           | 16          |
| 31. Fellowships .....                 | 16          |
| 32. Applications for Grants .....     | 17          |
| 33. Total Awards (1951-52) .....      | 21          |
| 34. Membership of the Committee ..... | 21          |
| 35. Appreciation of Service .....     | 23          |
| 36. Date of Next Meeting .....        | 23          |

Appendices

|              |                                                                                                                                                                   |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Appendix "A" | Investigation of methods for the use of methyl methacrylate in direct dental fillings, DR 25                                                                      |
| Appendix "B" | Adrenal cortical hormones and the growth of bones and teeth, DR 32                                                                                                |
| Appendix "C" | Cements for methacrylate inlays, DR 12                                                                                                                            |
| Appendix "D" | Experimental fusospirochetal infection with mixtures of pure cultures, DR 35                                                                                      |
| Appendix "E" | Chemical mechanisms in dental caries, DR 1                                                                                                                        |
| Appendix "F" | A study of nerve block, DR 31                                                                                                                                     |
| Appendix "G" | An investigation of the mechanism of repair of the supporting structures of the teeth (A), and Fusospirochetal infection and pre-existing inflammation (B), DR 22 |
| Appendix "H" | Development of a method for determining fluorine by means of a photo-electric colorimeter, DR 28                                                                  |
| Appendix "I" | The study of the closure of space following premature loss of deciduous teeth, DR 30                                                                              |
| Appendix "J" | A study of the calcifying potentialities of calcium oleate on the tissues of the dental pulp, DR 33                                                               |





TABLE OF CONTENTS (cont'd)

Appendices -

- Appendix "K" Studies of calcium metabolism in tooth with the aid of  $\text{Ca}^{45}$ , DR 34
- Appendix "L" Biological absorption of fluorine, DR 29
- Appendix "M" Fluoride content of enamel and dentine of teeth from Toronto children studied in relation to pabulum history of the children's diet, DR 13
- Appendix "N" A study of the normal and abnormal physiologic forces in the oral cavity, DR 26
- Appendix "O" Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radio-carbon, DR 23
- Appendix "P" A histological study of tooth germ development both intra- and extra-alveolar with a survey of pathological changes in the developing tooth, DR 27
- Appendix "Q" The effects of the physical consistency of food on the periodontium of the rat, DR 24
- Appendix "R" Suggested Research Projects (Min.4(x), 13th Mtg.)
- Appendix "S" Papers Published, Associate Committee on Dental Research.

Initial Distribution.

Apologies for their absence were received from -  
Dr. C. H. Best, Dr. J. B. Collip, Dr. J. E. W. Ferguson, Dr. J. B. Macdonald and Dr. R. H. McEwen.

2. Chairman's Remarks

THE CHAIRMAN welcomed Dr. Bagnall, who was attending his first meeting as a member of the Committee, and Mr. Christie, Dr. D'Iorio and Dr. Lussier who had come to report on their several projects. He said that Dr. Moyers had also been invited to be present and would arrive for the second session of the Committee to demonstrate some equipment which he is using under his grant. He also reported that he had spoken to Dr. Hunter and Dr. Williams but that neither of these two grantees was yet in a position to report to the Committee.





# NATIONAL RESEARCH COUNCIL

## Proceedings

### Thirteenth Meeting

#### of the

### ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,  
9:30 a.m., 8 - 9 March, 1951.

#### 1. Attendance

Dr. R. G. Ellis, Chairman  
Dr. J. S. Bagnall  
Dr. H. K. Brown  
Dr. E. Charron  
Dr. O. W. Ellis  
Dr. M. H. Garvin  
Dr. A. W. Ham  
Dr. H. R. MacLean  
Dr. J. J. Rae  
Dr. D. L. Thomson  
Col. E. M. Wansbrough

Mr. S. J. Cook, Secretary

#### By Invitation

Mr. D. R. Christie  
Dr. A. D'Iorio  
Dr. J. P. Lussier

Apologies for their absence were received from -  
Dr. C. H. Best, Dr. J. B. Collip, Dr. J.K.W. Ferguson, Dr. J.B. Macdonald and Dr. R. H. McDougall.

#### 2. Chairman's Remarks

THE CHAIRMAN welcomed Dr. Bagnall, who was attending his first meeting as a member of the Committee, and Mr. Christie, Dr. D'Iorio and Dr. Lussier who had come to report on their several projects. He said that Dr. Moyers had also been invited to be present and would arrive for the second session of the Committee to demonstrate some equipment which he is using under his grant. He also reported that he had spoken to Dr. Hunter and Dr. Williams but that neither of these two grantees was yet in a position to report to the Committee.





### 3. Minutes of the 12th Meeting

It was MOVED by Dr. Charron and SECONDED by Dr. Bagnall:

That the Minutes of the 12th Meeting having been  
circulated be taken as read and approved. CARRIED.

### 4. Business Arising out of the Minutes

#### (i) Report on Distribution of Bibliography on Caries Research (Min. 4(ii), 12th Mtg.)

THE SECRETARY presented a summary of the distribution of the Bibliography on Caries Research showing that 73 copies had been sold and 31 distributed on a complimentary basis making a total of 104 up to 1 March 1951. He said that copies had been sent to Australia, Canada, Denmark, England, New Zealand, Scotland, South Africa, and 62 copies had been distributed to institutions in twenty States of the American Union.

THE SECRETARY read a letter from Dr. Peter Adler, Assistant Professor of Orthodontics and Head of the Stomatological Clinic of the University, Debrecen, Hungary requesting a complimentary copy of the Bibliography. The letter pointed out the difficulty in securing quickly permission from the Hungarian Bank to pay for the book in Canadian currency, and offered to send in exchange the collected reprints of papers from that Dental School.

It was MOVED by Dr. Rae and SECONDED by Dr. Charron:

That a complimentary copy of the Bibliography be  
sent to Dr. Adler. CARRIED.

THE SECRETARY reported that three copies of the Bibliography had been sold to the Oregon State Dental School and that a request had been received for one further copy on a complimentary basis. On consideration, no action was taken in regard to this request.

#### (ii) Ownership of Copyright of the Bibliography on Caries Research (Min. 5, 12th Mtg.)

THE SECRETARY reported that Dr. Bagnall had very kindly made an assignment of his rights as author to the ownership of his Bibliography and that the copyright in this "published literary work" had been registered under serial no. 90302 in register of copyright no. 4, 9 December 1950, in the name of the National Research Council.

THE CHAIRMAN expressed the appreciation of the Committee to Dr. Bagnall for his gracious gesture in assigning the copyright of his monumental production to the Committee.







(iii) Correction in Report on DR 25 (Min. 15, 12th Mtg.)

THE SECRETARY reported that he had sent criticisms made by Dr. MacLean and Dr. Ham on this report to the Ontario Research Foundation and had received in reply the following comments:-

" Dr. MacLean's comment is quite correct. There is an error in the report which can be easily corrected by transposing two words. The sentence in question should read: 'Three curves are shown for Fastcrown, representing mixes of thick, medium and thin consistencies.'

" It is understood that Dr. Ham's comments were more in the nature of a constructive criticism. The histopathological analysis was made by a dentist who has had some experience in studying dogs' teeth and it is our belief that his findings are entirely reliable. If time permits, we shall arrange to have these results confirmed by a member of the histology department of the University of Toronto Dental School.

" To avoid possible confusion, each high-power photomicrograph in Report 5001, DR-21 might be more suitably labelled by the addition of the word 'showing'. Under figure 2, for example, the caption should possibly read '150X. Showing secondary dentine. Odontoblasts normal.' "

(iv) Correction in Report on DR 27 (Min. 17, 12th Mtg.)

THE SECRETARY said that in reply to a letter written to Dr. A.G. Nutlay, regarding the suggested error in the report on DR 27, he had been advised by Dean Bagnall that the line should read: "embedded in celloidin before decalcification."

(v) Reference to Report on DR 28 (Min.18, 12th Mtg.)

THE SECRETARY said he had inquired of Dr. MacLean whether a copy of Mr. Martin's thesis could be made available for loan to Dr. M.Doreen Smith but Dr. MacLean had replied that the worker now engaged in this project was rechecking on a number of points in the thesis and while she was making extremely good progress it did not appear that her thesis would be of much help to Dr. Smith at this time.

(vi) Status of Investigation under DR 29 (Min. 19, 12th Mtg.)

Consideration of this subject was deferred pending a review of the report on this project. (See Min. 17)

(vii) Preparation of Papers and Thesis, on DR 30 (Min.20,12th Mtg.)

THE SECRETARY said he had written to Dr. Walker regarding the possible preparation of two journal articles on this project and also for a copy of the thesis, on completion, that is being prepared by Dr.Liu.







THE CHAIRMAN Dr. Walker had replied that she thought this reference concerned DR 15 rather than DR 30, and added "It was in connection with DR 15 (A comparison of the patterns of eruption of the deciduous teeth in man with rates of growth of the dental arches) that the Committee discussed the preparation of two reports for publication. These are being prepared for DR 15 and will be ready by the end of this academic session."

(viii) New Members (Min. 34 (ii), (iii), 12th Mtg.)

THE SECRETARY reported that the National Research Council had approved the recommendations made by the Committee regarding the appointment of Dr. J. B. Macdonald vice Dr. Stewart and Dr. J. S. Bagnall vice Dr. Box, and that these two new members had been notified accordingly.

THE CHAIRMAN welcomed Dr. Bagnall and expressed regret that Dr. Macdonald had been unable to attend because of illness.

(ix) Fellowship Application - Dr. L. A. Gill (Min. 36, 12th Mtg.)

THE SECRETARY said that he had received a letter from Dean Mowry in which it was stated that after talking the matter over with Dr. Charron, it had been found that the proposed work might possibly be better executed under a grant instead of a fellowship; particulars regarding grants had been sent to Dr. Mowry.

DR. THOMSON said that he understood Dr. L. A. Gill now had an appointment which would not permit him to undertake the proposed work.

(x) Requests for Suggestions re New Research Projects (Min. 37, 12th Mtg.)

THE SECRETARY said that in accordance with the Committee's instructions he had sent a letter on 10 November 1950 to all members of the Committee inviting them to furnish a list of proposals on which in their opinion profitable research in the field of the dental sciences might be done.

He had been informed that Dr. McDougall had sent this request to every member of the dental profession in British Columbia. No definite suggestions had been received from any of the members of the Committee or from any of the British Columbia dentists circularized by Dr. McDougall.

DR. GARVIN reminded the Committee that he had presented two items at a previous meeting of the Committee.

THE CHAIRMAN said that the Secretary would publish a list as a continuous appendix to the proceedings in which suggestions from the members could be reported and that the list would be started with the two items mentioned by Dr. Garvin.

APPENDIX "R"

DR. RAE asked whether it would be worthwhile to extend the plan followed by Dr. McDougall to the whole Dominion.







THE CHAIRMAN said he thought such a procedure might present difficulties in that suggestions might be received which were impracticable and the sponsors of such ideas might feel unkindly towards the Committee when their ideas were not put into effect.

DR. CHARRON said he thought individual contacts by members of the Committee would be more profitable but it would be interesting to know whether Dr. McDougall had received any response to his circular letter.

#### Consideration of Reports from Grantees

##### 5. DR 1 - Dr. J. J. Rae

###### "Chemical Mechanisms in Dental Caries"

DR. RAE presented a summary and interim report No. 11 on his project, both of which appear in APPENDIX "E"

DR. LUSSIER said he thought this project presented for the first time evidence that ammonia production increases with glucose.

DR. RAE said that they were measuring not the content but the ammonia produced at 37°C.

##### 6. DR 2 - Dr. G.H.W. Lucas

###### "A Study of Alveolar Oxygen Tension and Blood Oxygen Content during Nitrous Oxide Anaesthesia"

No report was received under this project.

##### 7. DR 12 - Dr. A.E.R. Westman

###### "Cements for Methacrylate Inlays"

A summary report by Dr. Westman presented by Mr. D.R. Christie appears in APPENDIX "C"

##### 8. DR 13 - Dr. M. Doreen Smith

###### "Fluoride Content of Enamel and Dentine of Teeth from Toronto Children Studied in Relation to Pabulum History of the Children's Diet"

A summary report was read and the complete report was examined. Both appear in APPENDIX "M"

In paragraph 3 of the summary, the fluorine content of Toronto water was recorded as "0.1%F". The Secretary was asked to question this item as it was thought it should have been reported in p.p.m.





9. DR 15 - Dr. Norma Ford Walker

"A Comparison of the Patterns of Eruption of the Deciduous Teeth in Man with Rates of Growth of the Dental Arches"

DR. WALKER reported in a letter dated 7 February 1951 that "the report of DR 15 is now in the form of the first draft of a thesis and is being read by the special committee appointed to supervise the work of Mrs. Margaret Hatton. When the final draft is ready, a copy will be sent to your Committee."

10. DR 22- Dr. C. H. Best

- A. "An Investigation of the Mechanism of Repair of the Supporting Structures of the Teeth"
- B. "Fusospirochetal Infection and Pre-existing Inflammation"

A report by Dr. C. H. Best on each of the two parts of this project appears in APPENDIX "G"

DR. GARVIN questioned the item under part A(2.) Surgical approach, which states (last line of para. 1) "This has never been established". He said that while this might be true from the clinical standpoint there was work on record concerning the formation of cementum and bone in such an environment.

There was some discussion regarding the two phases of the project and the question was raised as to why they were not carried on under separate grants.

THE CHAIRMAN read from the proceedings of the 11th Meeting (page 14) noting that two applications received last year from Dr. Best had been combined and that it had been suggested to the grantee that the Committee expected him to proceed with the work on fusospirochetal infection as well as on part A of the investigation.

In the new application under this grant, for which a total of \$5,000 is being asked, it was noted that the outline of Dr. W.J. Linghorne's work under this project was described as follows:-

" An experimental method of obtaining functional reattachment of the soft tissues to the tooth and the regeneration of alveolar bone has been developed and the physiologic principles involved in the reparative process are being studied. In addition a study is being carried out to ascertain if this regenerative ability of the periodontal structures has a place in the physiology of these tissues. It is hoped that these studies will provide a physiologic basis for the development of clinical procedures involving the regeneration of the supporting structures of the teeth."

DR. RAE said that he had read the report but he could not find the results of the year's work in it. He found references to "approaches" and "plans" and the statement that "results" are encouraging but he could find no statement of the results obtained.







THE CHAIRMAN remarked that this work by Dr. Linghorne is being carried on in a very favourable environment.

DR. A. W. HAM added that Dr. Linghorne has two basic problems, and that both require a great deal of work to be done for their solution.

DR. GARVIN said the report would be more convincing if some chips had been used on humans.

11. DR 23 - Dr. C. P. Leblond

"Entry of Phosphate and Carbonate Salts into Teeth as shown with the Help of Radio-phosphorus and Radio-carbon"

A report by Dr. Leblond appears in

APPENDIX "O"

THE CHAIRMAN suggested that the Committee might ask Dr. Leblond and Dr. Bélanger to attend the next meeting to report on this project.

12. DR 24 - Dr. C.H.M. Williams

"The Effects of the Physical Consistency of Food on the Periodontium of the Rat"

A summary report by Dr. Williams appears in APPENDIX "Q"

A bound copy of three reports submitted by Dr. Williams was passed to the members for examination as it had not been duplicated for distribution. The titles of the three papers contained in this report under the name of Dr. D.G. Watt are as follows: (i) "The effects of the physical consistency of food on the periodontium of the rat"; (ii) "Preliminary observations on the effects of the physical consistency of food on the width of maxilla of the rat"; (iii) "The effects of the physical consistency of food on the size and form of the mandible of the adult rat".

It was noted in the report of the grantee that when the data have been fully organized for presentation, a copy of the complete report will be submitted to the Committee.

In the meantime it was agreed that the bound copy of three papers referred to above need not be reproduced in these proceedings but merely be put on file for reference.

13. DR 25- Dr. A.E.R. Westman

"Investigation of Method for the use of Methyl Methacrylate in Direct Dental Fillings"





A summary and reports Nos. 5003 and 5101 by Dr. Westman were presented by Mr. D. R. Christie. These reports appear in  
APPENDIX "A"

MR. CHRISTIE said that he had also tried to determine adhesion to dentine using cattle ribs. On small samples the degree of adhesion was found to be about the same as that to glass.

THE CHAIRMAN said this was a very interesting and practical project.

DR. GARVIN was not quite clear as to what takes place during the first hour in which the compression is kept on. He asked whether the shrinkage would have been different if the pressure had been released and added that he did not think it was practical to keep fillings dry for one hour.

MR. CHRISTIE said that polymerization was fairly complete in ten minutes but shrinkage continued on a decreasing scale. However, after the initial setting had taken place any further shrinkage should be only on the surface.

DR. RAE inquired whether zinc oxyphosphate cements were used under the fillings.

THE CHAIRMAN said this was not always done.

DR. GARVIN said that in Pennsylvania they had stopped using Kadon and had suggested using zinc oxyphosphate cements.

MR. CHRISTIE recalled that a German paper he had read recommended that a lining material should be used in all cases.

DR. GARVIN inquired whether any work had been done on the use of protective varnishes on the pulp.

MR. CHRISTIE, No.

DR. MACLEAN observed that in their school they felt there was some danger to pulp in the use of methacrylate fillings. He said cement bases were needed. They had stopped using methacrylates.

DR. GARVIN reported that many methacrylate fillings were used in Winnipeg, that they were harder than the ordinary fillings and were really very wonderful although some of them did fall out through shrinkage.

THE CHAIRMAN said these fillings probably showed greater adhesion to the tooth than was generally supposed and that any breakage probably arose from the rather common practice of interfering with the filling before it was properly set.

MR. CHRISTIE said that a well rounded undercut was necessary in order to secure a proper cure of the filling.







DR. O.W. ELLIS suggested that the use of a zinc base would reduce the area of contact between the filling and the dentine.

DR. CHARRON mentioned that when the manufacturers' instructions regarding techniques are followed exactly, good results are invariably secured.

COL. WANSBROUGH agreed.

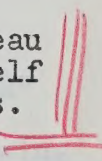
DR. O.W. ELLIS asked that a minor change be made in page A3 of report 5101 ( last para.) so that it would read as follows:-

" 'Volan' is not recommended by the manufacturers as a dental material etc ...."

MR. CHRISTIE said that experiments were carried out at room temperature. He hoped to prepare a paper shortly on this work for publication.

THE CHAIRMAN said that no subject was more timely. He thought that useful comments had been made in this discussion and he was sure that everyone present regretted that Dr. Westman, the grantee, had been confined to hospital and therefore unable to attend this meeting himself. However, Mr. Christie had made an excellent presentation.

It was AGREED to send a message to Dr. Westman from the Committee expressing the hope that he would have a speedy recovery from his present illness.

THE CHAIRMAN said that the Executive was of the opinion that it would be useful for Mr. D.R. Christie to visit the National Bureau of Standards at Washington for about a week to familiarize himself with the work that was being done there on methacrylate fillings. It was believed that such a visit would serve to correlate the work in this field between the National Bureau of Standards and the project being sponsored by the Committee at the Ontario Research Foundation. 

The Committee AGREED.

#### 14. DR26 - Dr. R. E. Moyers

"A Study of the Normal and Abnormal Physiologic Forces in the Oral Cavity"

A report by Dr. Moyers (presented at the Second Session) appears in APPENDIX "N"

THE CHAIRMAN said that Dr. Moyers had needed a small additional amount to complete the purchase of his equipment and that under date of 13 December 1950 he had authorized the award of \$10.00 additional under DR 26 making the total for the year \$160 (grant listed in Min. 39, 11th Mtg.).







DR. MOYERS accompanied by Dr. E.E. Johns a staff member of the Faculty of Dentistry, and Dr. Hult registered in the school of Graduate Studies, University of Toronto, jointly demonstrated a strain-gauge equipment used in this project for the measurement of forces in orthodontic work. The strain gauges employed are applied to each tooth in the arch and it is thus possible to separate and measure the forces imposed by orthodontic appliances to an accuracy of 1/15000th of an inch.

THE CHAIRMAN complimented the three demonstrators on their work. He said that the equipment used and shown to the members had been bought out of funds provided under this grant and would continue to be used for many years by graduate students. Thus, while no application for a further grant is being made for 1951-52, the influence of graduate work resulting from the continued use of this new equipment would be felt for a long time.

15. DR 27 - Dr. A. G. Nutlay

"A Histological Study of Tooth Germ Development both Intra- and Extra-alveolar with a Survey of Pathological Changes in the Developing Tooth"

A summary of work done under this project appears in

APPENDIX "P"

16. DR 28 - Dr. O. J. Walker

"Development of a Method for Determining Fluorine by means of a Photo-electric Colorimeter"

A summary and report on this project appear in

APPENDIX "H"

Supplementing the report, the Chairman read the following outline of the research that is proposed to be done under this grant in 1951-52:

" This research has been in progress for some years and during the last two years it has been supported by the Associate Committee on Dental Research of the National Research Council. Up to the present time it has been possible to develop a rapid method for both fluorides and phosphates in water in the presence of interfering ions. These methods are satisfactory for most interfering elements but the fluoride method can only be used when phosphates less than 6 p.p.m. are present. Several other methods for fluorides have been studied. One of these involves the fading of a zirconium-hematoxylin lake, but this is unsatisfactory for fluorides less than 10 p.p.m. Much more promising results have been received in the investigation in which one makes use of the fading of an aluminum-hematoxylin lake. It is proposed to continue with the investigation of this method. Efforts are also to be made to adapt the methods for fluorides







and phosphates to the analysis of bone, teeth, and other organic materials. It is expected that the investigation will be completed in 1951-52. The objective of the research is to provide a rapid method for determining fluorides in water and in other materials."

17. DR 29 - Dr. M. Doreen Smith

"Biological Absorption of Fluorine"

A report on this subject appears in APPENDIX "L"

Copies of the reprint mentioned in the first paragraph of the report are on file in the office of the Secretary of the Committee.

DR. D.L. THOMSON said that he had read the paper with care but he thought that if the analytical methods were such as to permit a variation of 100% in the results, the conclusions to be drawn from the work would not be very useful.

THE CHAIRMAN said that this project had now passed out of the hands of the Committee and the work was being continued as material for a Ph.D. thesis without financial support from this Committee.

18. DR 30 - Dr. Norma Ford Walker

"The Study of the Closure of Space following Premature Loss of Deciduous Teeth"

A summary report appears in APPENDIX "I"

A more detailed report of this study will be presented at the next meeting of the Committee.

19. DR 31 - Dr. L.B. Jaques and Mr. G.J. Millar

"A Study of Nerve Block"

A report on this project appears in APPENDIX "F"

DR. GARVIN thought that failure was either due to the use of wrong anaesthetics or to poor technique.

DR. THOMSON said that the progress made seemed rather slow.

THE CHAIRMAN read the following extracts from the résumé of present state of knowledge of this project as submitted by the applicants:  
"Inability to produce satisfactory block of the inferior dental nerve with procaine hydrochloride is a frequent experience in dental surgery. This problem has been drawn to our attention by Dr. M.J. MacDonell of Saskatoon, who raised the question recently.





The phenomenon has been explained as being due to faulty technique or to the presence of an accessory nerve supply involving branches of the trigeminal, facial or autonomic nerves, but it seems to be agreed that the presence of inflammation involving the pulp or the tissue surrounding the roots is a condition which is most likely to lead to an unsuccessful block.

" It is proposed (1) to determine, in the cadaver, the probable length of human inferior dental nerve which is contacted by the anaesthetic when a conventional injection is made.

(2) to determine the length of a mixed nerve which must be anaesthetized in order to block impulses from normal pain endings and from pain endings in an inflamed area.

(3) to study the frequency of the impulses from normal and "inflamed" pain endings and to determine whether summation of the extrinsic potentials past a blocked area can be observed when the frequency of the impulses arriving at the block is high.

(4) to investigate the possibility of modifying the conventional injection technique to give satisfactory nerve block in difficult cases."

The report was accepted.

20. DR 32 - Dr. A. W. Ham

"Adrenal Cortical Hormones and the Growth of Bones and Teeth"

A summary report presented by Dr. Ham appears in  
APPENDIX "B"

21. DR 33 - Dr. H. A. Hunter

"The Investigation of the Mechanism Concerned in the Deposition of Lime Salts formed in Bridging over a Pulp Exposure"

The title of this project has been changed and broadened as indicated above.

A report on the project appears in  
APPENDIX "J"

DR. CHARRON said that Dr. J.A. Renaud, at the preclinic and Department of Operative Dentistry, University of Montreal, now has 800 cases of exposed pulps which he is studying. Dr. Charron said that he would put Dr. Renaud in touch with Dr. Hunter.

22. DR 34 - Dr. A. D'Iorio

"Studies of Calcium Metabolism in Tooth with the Aid of  $Ca^{45}$ "

A summary and report appear in

APPENDIX "K"







These two documents were presented and discussed by Dr. D'Iorio and Dr. J.P. Lussier, and the latter speaker outlined the further stages of work that are planned for this study. It is proposed to investigate calcium diffusibility through tissues and enamel.

DR. THOMSON inquired whether it would be possible to use much smaller quantities of calcium of tracer quality.

DR. LUSSIER said they had tried calcium of 800 gamma.

DR. HAM wondered why the investigators who were concerned with the turnover of calcium should use a growing animal.

DR. THOMSON said that the report shows the most surprising thing, namely, that even though growing animals were used, the calcium turnover was remarkable.

DR. RAE inquired about the influence of fluorine and Dr. Thomson suggested that Dr. Lussier might consult Dr. M. Doreen Smith and arrange to obtain fluorine analyses.

DR. D'Iorio drew attention to corrections that should be made on page K-2 para. 1 under the table:

In the second line for "25 ml. a ml." read "25 ml. 4 ml. are diluted..."

In line 5 for "9.5 ml." read "0.5 ml."

Para. 2 line 3 for "100 wire screen" read "100-mesh wire screen"

THE SECRETARY said these corrections would be made.

THE CHAIRMAN thanked Dr. D'Iorio and Dr. Lussier for their presentation and wished them success in their future work.

23. DR 35 - Dr. J. B. Macdonald

"Experimental Fusospirochetal Infection with Mixtures of Pure Cultures"

A summary and report appear in

APPENDIX "D"

THE CHAIRMAN expressed regret that illness had prevented Dr. J.B. Macdonald from attending this meeting and presenting his own report.

#### 24. Finances

THE SECRETARY presented a report on the finances of the Committee as of 31 January 1951 which showed that of the total sum made available to the Committee for the year namely \$35,500, the amount of expenditures was \$28,507.14 and commitments \$4,760.06 leaving







a free balance of \$1584.37 to which might be added a total of \$640 to be returned by grantees who found that this amount would be surplus to their requirements during the present year. This made \$2224. available to the Committee at the present time from funds expendable prior to 31 March 1951.

25. Air Travel (1951-52)

It was MOVED by Dr. Thomson and SECONDED by Dr. Ham

That travel by air be authorized for 1951-52 for Dr. M. H. Garvin, Dr. H. R. MacLean and Dr. R. H. McDougall and that the Executive be empowered to deal with any further cases of air travel as they may arise during 1951-52. CARRIED.

26. New Business

(i) Appraisal of Applications

It was MOVED by Dr. Ham and SECONDED by Dr. Thomson

That if the Chairman sees fit, outside opinions may be secured in advance of consideration by the Committee of both renewals and new applications for grants. CARRIED.

(ii) News Items

THE CHAIRMAN asked Dr. Garvin if the news items supplied to him by the Secretary provided satisfactory coverage of the meetings for publication in the Journal of the Canadian Dental Association.

DR. GARVIN said that material received from the Secretary was quite satisfactory in every respect. His only difficulty had been to determine whether such information should be included under News Items in which case the deadline was the 10th of the month, or whether he should put these reports in the Manuscript Section in which event they should be in his hands by the third week of the month preceding publication.

THE CHAIRMAN said that he thought these news reports should be published in the News Items section of the Journal.

(iii) South African Dental Committee

THE SECRETARY read a letter from the Secretary Treasurer, Council for Scientific and Industrial Research of South Africa, dated 17 February 1951 pointing out that several national committees and subcommittees have been formed including one on Dental Diseases which is occupied mainly with questions of dental and oral interest.





The members of this Subcommittee are as follows:-

Prof. J. C. Middleton Shaw, Chairman  
Prof. J. T. Irving  
Dr. P. J. du Toit  
Dr. E. H. Cluver  
Dr. J. Staz  
Dr. T. Ockerse  
Dr. B. Cohen  
Dr. W. A. Odendaal  
Dr. A. J. Clement and  
Prof. S. F. Oosthuizen

" The terms of reference of the subcommittee on Dental Diseases are to survey the field of dental research and to advise the Council through the Medical and Dental Research Committee on how best to ensure its proper development in South Africa."

THE SECRETARY said that a reply had been made offering the co-operation of the Associate Committee on Dental Research. A complimentary copy of Dr. Bagnall's Bibliography had also been sent.

DR. BAGNALL said that several of the men whose names appeared in the list as members of the Subcommittee had made interesting contributions in the field of dental research.

#### 27. Visitors at the Next Meeting

THE CHAIRMAN pointed out that five grantees had been invited to attend this meeting and asked for suggestions regarding grantees who might be invited to present reports in person at the next meeting.

DR. THOMSON suggested and it was AGREED that Dr. Hunter, Dr. Leblond, Dr. Williams and possibly Dr. Linghorne be invited.

The Meeting adjourned at 4 p.m. and the 10th meeting of the Executive was immediately convened.

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#### SECOND SESSION

The Committee resumed at 9:30 a.m., Friday 9 March, 1951, in the Council Chamber.

#### 28. Attendance

Dr. R.G. Ellis, Chairman  
Dr. J.S. Bagnall  
Dr. H. K. Brown  
Dr. E. Charron  
Dr. O.W. Ellis  
Dr. M.H. Garvin  
Dr. A. W. Ham  
Dr. H.R. MacLean  
Dr. J.J. Rae  
Dr. D.L. Thomson  
Col. E.M. Wansbrough





28. Attendance (continued)

Mr. S. J. Cook, Secretary

By Invitation

Dr. R. E. Moyers  
Dr. E. E. Johns  
Dr. Hult

29. Demonstration by Dr. Moyers

DR. MOYERS and his two assistants demonstrated their equipment (a report on this appears in Min. 14 of these proceedings).

30. Executive Meeting

THE CHAIRMAN reported that the Executive had met for two hours on the preceding day and their recommendations would be submitted to the Committee under the relevant items on the Agenda.

31. Fellowships

Two applications for Fellowships had been received and both had been recommended for award by the Executive. The applications were as follows:

(i) Dr. Gerald Shklar

Oral Pathology and Bacteriology

Place of Research: Tufts College Dental School, under Dr. Irving Glickman. Amount of award recommended \$1500.

(ii) Dr. Willa L. Liu (Chou)

A Study of Migration of Tooth Germs before and during Mixed Dentition.

This work will be carried on in the Department of Orthodontics, Faculty of Dentistry, University of Toronto.

Amount of award recommended \$2200.

It was MOVED by Dr. Thomson and SECONDED by Dr. O. W. Ellis,

That the recommendations of the Executive be approved and fellowships awarded to Dr. Shklar and Dr. Liu. CARRIED.





### 32. Applications for Grants

THE CHAIRMAN said that all applications for grants for 1951-52 had been carefully reviewed by the Executive and recommendations had been made regarding the amounts of the awards to be granted. As each application was considered he would present the recommendations of the Committee. Action was then taken in regard to the applications for renewals and new applications as recorded hereunder:

#### Renewals

| <u>Grant No.</u> | <u>Grantee</u>      | <u>Amount</u> (1951-52) |
|------------------|---------------------|-------------------------|
| DR 1             | Dr. J. J. Rae       | \$<br>960               |
| DR 12 and 25     | Dr. A.E.R. Westman  | 4,000                   |
| DR 13            | Dr. M. Doreen Smith | 1,600                   |

Dr. Bagnall said he would like to see a more specific project. The question now is to find whether analyses give significant results. If control is to be adopted it would be a long-term problem.

|       |                |       |
|-------|----------------|-------|
| DR 22 | Dr. C. H. Best | 2,300 |
|-------|----------------|-------|

THE CHAIRMAN said that the application was for \$5000 as compared with \$3800 in the preceding year when two applications had been combined into one. The Executive recommended continued support of the work being done by Dr. Linghorne on repair and attachment but were inclined to be critical of the grant in support of the bacteriological side of the study being carried on by Mr. O'Connell. The Executive recommended that a further unstated amount be granted, after consultation by the Chairman, the total of the grant not to exceed \$5,000.

Dr. RAE thought there were two separate projects and doubted that Mr. O'Connell should be supported by this Committee, since he is a bacteriologist on the University staff. He thought the Committee's funds should only be used for the payment of technical assistants and equipment.





| <u>Grant No.</u>                                  | <u>Grantee</u>                                                                                                                                                                                                                                                                                                                                                                                         | <u>Amount</u> |
|---------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
|                                                   | DR. THOMSON thought it was not unreasonable to give a research director assistance by way of a grant.                                                                                                                                                                                                                                                                                                  |               |
| DR 23                                             | Dr. C. P. Leblond                                                                                                                                                                                                                                                                                                                                                                                      | 2400          |
|                                                   | The application was for \$2400 and it was AGREED to grant Dr. Leblond \$200 additional under his 1950-51 grant for the purchase of items that might be bought before 31 March 1951, and to increase his grant by this amount in 1951-52 if he was not able to spend the \$200 before 31 Mar. 1951. (This turned out to be the case so the award for 1951-52 is in the full amount of the application). |               |
| DR 28                                             | Dr. O. J. Walker                                                                                                                                                                                                                                                                                                                                                                                       | 1000          |
| DR 31                                             | Dr. L.B. Jaques and Mr. G.J. Millar                                                                                                                                                                                                                                                                                                                                                                    | -             |
|                                                   | The application for \$2000 was deferred pending consultation by the Chairman.                                                                                                                                                                                                                                                                                                                          |               |
| DR 33                                             | Dr. H. A. Hunter                                                                                                                                                                                                                                                                                                                                                                                       | 1600          |
|                                                   | Title changed to "The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure"                                                                                                                                                                                                                                                                |               |
| DR 34                                             | Dr. J. P. Lussier (formerly in the name of Dr. A.D'Iorio)                                                                                                                                                                                                                                                                                                                                              | 350           |
|                                                   | It was AGREED to grant to Dr. Lussier \$850 in 1950-51 for the purchase of equipment that can be bought before 31 March 1951, and to increase his grant by this amount in 1951-52 if he is not able to spend the \$850 before 31 March 1951.                                                                                                                                                           |               |
| DR 35                                             | Dr. J. B. Macdonald                                                                                                                                                                                                                                                                                                                                                                                    | 2600          |
|                                                   | It was AGREED to make an additional grant of \$200 under this project from 1950-51 funds for the purchase of equipment that can be bought before 31 March 1951 and, as before, to carry the amount forward to 1951-52 if necessary.                                                                                                                                                                    |               |
| Total amounts granted under renewal applications: |                                                                                                                                                                                                                                                                                                                                                                                                        |               |
| (i) from 1950-51 funds - \$1050                   |                                                                                                                                                                                                                                                                                                                                                                                                        |               |
| (ii) from 1951-52 funds - \$16,810                |                                                                                                                                                                                                                                                                                                                                                                                                        |               |





New Applications

| <u>Grant No.</u>        | <u>Grantee</u>                                                                                                                       | <u>Short Title</u>                                                                      | <u>Amount</u> |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|---------------|
| DR 36 ✓                 | Dr. Gordon Nikiforuk,<br>Assoc. Prof. of Pedo-<br>dodontics, Dept. of<br>Dental Research,<br>University of Toronto,<br>Toronto, Ont. | Demineralization of<br>hard tissues as bone<br>and teeth by organic<br>chelating agents | \$<br>2400    |
| DR 37 ✓                 | Dr. R.E. Moyers<br>Prof. and Head,<br>Dept. of Orthodontics,<br>Faculty of Dentistry,<br>University of Toronto,<br>Toronto, Ont.     | Further studies in the<br>electromyography of<br>the head, face and neck.               | 960           |
| DR 38 ✓                 | Dr. Kenneth J. Paynter,<br>Faculty of Dentistry,<br>University of Toronto,<br>Toronto, Ont.                                          | The effect of Thiouracil 1850<br>on the development of<br>molar teeth of rats.          |               |
| (i) Dr. F. Hugh Lawford | Technical Director,<br>Consumers' Research Laboratories, Ltd.,<br>Toronto, Ont.                                                      | The effect of carbo-<br>hydrate on dental caries.                                       |               |

THE CHAIRMAN said that \$2500 was requested under this grant. There had been considerable discussion in the Executive regarding this application because it had come from an industrial organization. Dr. Lawford, the Director of Consumers' Research Laboratories, holds a Ph.D. from the University of Toronto, and prior to his present position was associated for ten years with Standard Brands Limited. In his covering letter Dr. Lawford had written:

" Consumers' Research Laboratories Limited was organized in November 1947 and financed entirely with Canadian capital. The staff are engaged solely in scientific research and development. The prime objectives, as set out in Letters Patent are,-

" To establish, maintain and operate laboratories for technical, industrial and other scientific research and to carry on and engage in research and development work "

" The Laboratories are operated essentially as a non-profit organization and copies of Annual Balance Sheets will be supplied to you upon request.

" In order to attract and retain fully qualified research scientists, the administrative policy includes in the annual budget an assignment for the specific purpose of academic exploratory research, free of any industrial or commercial support or encumbrance. It is expected results of such assignments will be published in the usual scientific periodicals.





" Owing to the magnitude of research already completed on food and nutrition problems, it was decided that the first major academic project should be restricted to a field of related endeavour. Miss Ballantyne, as Research Associate, was assigned a problem in connection with dental caries. This work is under my immediate direction with the advisory counsel of Prof. Rae of the University of Toronto. All expenses for this research have been supplied from current operations in conformity with the administrative policy described above."

DR. RAE, with whom Miss Ballantyne had worked on his project, spoke in support of the application. He said that Dr. Lawford felt that the Consumers' Research Laboratories Limited was similar to the Ontario Research Foundation in its general plan.

DR. THOMSON pointed out that the making of grants to non-university organization, while it should not be excluded, was on a different basis from working with a university. It was, therefore, necessary to scrutinize the project carefully and to consider whether the job should be done and whether the proposed industrial organization was the place in which this project should be carried out.

DR. SAUNDERSON suggested that the problem might be referred to Assisted Researches Committee of the National Research Council for a decision as to policy.

THE CHAIRMAN thought the decision should rest on (i) nature of the problem, (ii) whether there was more than the average hope that the proposed organization was competent to do the job.

DR. THOMSON suggested that the grant might be made to Dr. Rae on the understanding that he might ask that Miss Ballantyne be given leave of absence to work as his assistant on a part-time basis.

It was MOVED by Dr. O.W. Ellis and SECONDED by Dr. Garvin,

That full information regarding this application be sent to the Assisted Researches Committee for a decision as to N.R.C. policy in relation to such a grant.

CARRIED.

THE CHAIRMAN said that if the decision from the Assisted Researches Committee were not favourable, the Executive might consider an alternative proposal.

AGREED.

(ii) Dr. H. A. Hunter  
Assoc. Prof. of Dentistry,  
Faculty of Dentistry,  
University of Toronto,  
Toronto, Ont.

A histological study of  
the teeth and jaws of  
rats placed on a vitamin  
B-complex-deficient diet





THE EXECUTIVE had decided that insufficient information had been supplied on this application, and recommended that additional data be obtained before a decision is reached.

It was AGREED to enquire of Dr. Hunter (i) whether he planned to feed animals on a B-Complex-deficient diet; (ii) what was his lead for the problem and why does he want to do it? (iii) to suggest that the day is past when the B-complex needs study; (iv) to point out that there was more interest in the bacteriological aspects than in the histologic study. AGREED  
(Application withdrawn 4 April 1951)

### 33. Total Awards (1951-52)

It was MOVED by Dr. Charron and SECONDED by Dr. MacLean,

That the action taken in respect of all grants as outlined in these proceedings be approved as follows:

|                   | <u>1950-51 funds</u> | <u>1951-52 funds</u> |
|-------------------|----------------------|----------------------|
|                   | \$                   | \$                   |
| Additional grants | 1050                 |                      |
| Renewal grants    |                      | 16,810               |
| New applications  |                      | <u>5,210</u>         |
|                   |                      | 22,020               |
| Fellowships       |                      | <u>3,700</u>         |
| Total             | <u>1050</u>          | <u>25,720</u>        |

### 34. Membership of the Committee

THE CHAIRMAN reported that four members of the Committee would complete their second term of office on 31 March 1951, and it was the general rule in committee memberships that members having served two terms should drop out for at least one year before being eligible for new appointments.

Under this plan four members would retire on 31 March 1951 - namely - Dr. R. G. Ellis, Dr. E. Charron, Dr. O. W. Ellis and Dr. J.K.W. Ferguson. The partial term of Dr. J. B. Macdonald, appointed in place of Dr. Box, would also expire on the same date.

President Mackenzie had said that while the rule of non-renewal of a membership following a two-term tenure was a sound procedure in general, he thought that the Committee should be careful not to lose the continuity of dental direction of the Associate Committee on Dental Research. He therefore recommended that consideration be given to the nomination of Dr. R.G. Ellis and Dr. E. Charron for a further term of three years.





He pointed out that the Associate Committee on Dental Research is a comparatively new organization, and that it is functioning extremely well under its present direction. He saw no objection to applying the rules of membership in the case of members of the Committee who represent the basic sciences, but he felt strongly that the dental direction of this Committee should be retained. He pointed out that both Dr. R.G. Ellis and Dr. E. Charron represent important sections of the dental profession and he said he was sure that the National Research Council would be very pleased to receive a recommendation that both of these members should be asked to serve at least one further term.

THE EXECUTIVE made the following recommendations:-

(i) That Dr. R. G. Ellis and Dr. E. Charron be nominated for a further term of three years each from 1 April 1951;

(ii) That the following be nominated for a term of three years from 1 April 1951:

Dr. A. C. Burton, Professor and Head of the Department of Biophysics, University of Western Ontario, London, Ont. (in succession to Dr. O. W. Ellis).

Dr. J.D. Hamilton, Professor of Pathology, Queen's University, Kingston, Ont. (in succession to Dr. J.K.W. Ferguson)

Dr. J.B. Macdonald, Assistant Professor of Bacteriology, University of Toronto, Toronto, Ont. (who has served a partial term in succession to Dr. C.B. Stewart)

THE CHAIRMAN said that the Committee owed a great debt of gratitude to Dr. O.W. Ellis and Dr. J.K.W. Ferguson for their great contribution to the Committee during their term of office.

This announcement was received with applause.

It was MOVED by Dr. Rae and SECONDED by Dr. Bagnall,

That the recommendations of the Executive in regard to changes in Committee membership as outlined above be approved.

CARRIED.

THE CHAIRMAN suggested that as the Department of National Health and the Royal Canadian Dental Corps were represented by ex officio members on the Committee, he thought it would be in order to add the Director of Dental Services, Department of Veterans Affairs also as an ex officio member since the D.V.A. has now a large dental group on the staff.







It was MOVED by Col. Wansbrough and SECONDED by Dr. Brown,

That the Associate Committee on Dental Research recommend to the National Research Council that the Director of Dental Services, Department of Veterans' Affairs be made a member ex officio of the Dental Committee

CARRIED.

35. Appreciation of Service

DR. O.W. ELLIS said that he would like to express his pleasure and satisfaction at having been privileged to serve two terms as a member of the Associate Committee on Dental Research. The programs of the Committee had always held items of special interest for him and he had enjoyed entering into the discussions on Committee projects especially when these touched on metallurgical problems. He felt it had been an honour to be associated with those who were giving such excellent direction to dental research in this Dominion and he wished the Committee continued success in its work.

THE CHAIRMAN said all members of the Committee were very grateful to Dr. O. W. Ellis and to Dr. J.K.W. Ferguson for their advice and assistance and he asked the Secretary to send to these two retiring members letters expressing appreciation of the Committee for their services.

36. Date of Next Meeting (Min. 40(i), 12th Mtg.)

THE CHAIRMAN said that it had been decided to hold the next meeting 26-27 September, 1951, but that it now appeared preferable to change these dates to 27-28 September 1951. There was a possibility that the Committee might be able to complete its business on the first of these two days.

AGREED.

The Meeting adjourned at 12:45 p.m.

A. E. R. Westman  
Signature

Date: 31 January, 1951.





APPENDIX "A"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951

Subject: Investigation of methods for the use of methyl methacrylate in direct dental fillings

Grantee: Dr. A.E.R. Westman

Project No. DR 25      Amount of Grant: (\$3,000 includes DR 12)

SUMMARY OF WORK

Since the last meeting of the Associate Committee investigations have been made on volumetric shrinkages of acrylic filling materials in confined spaces similar to tooth cavities, and also on the related matter of adhesion of acrylic filling materials. Detailed reports on these topics will be found in our progress reports which have been submitted to the Committee.

The results of this work indicate that adhesion of methyl methacrylate filling materials to tooth structure should be good at least during the period of contraction, and in such a case, volumetric shrinkage can be controlled by being localized to the free surface of the filling.

Dry conditions are indicated for the preparation of cavities and consideration should also be given to ensure that matrices are actually non-adherent to the acrylic filling materials.

A paper, based on the research of this project, is being completed for publication in the Journal of the Canadian Dental Association.

Date: 31 January, 1951.

A. E. R. Westman  
Signature





## APPENDIX "A"

### ONTARIO RESEARCH FOUNDATION

Department of Chemistry

### Dental Materials Research Fellowship

Report 5003 - DR 25

#### Scope of this Report

An investigation has been made of the volumetric shrinkages of Kadon, Fastcrown and Replica in closed molds in the same manner that fillings would be placed in teeth. The extent of the shrinkage and, equally important, the region of the shrinkage, indicate what may be expected of these filling materials in practice.

#### Determination of Shrinkage

First attempts at measuring volumetric shrinkages in a closed mold were not satisfactory because apparently too large a sample of acrylic filling material was used. A two-part, steel mold in the shape of a frustrum of a cone with a top diameter of one inch, a vertical angle of  $20^\circ$  and a height of 0.4 inches gave specimens which consistently showed extensive internal porosity. When the height of the mold was reduced to 0.1 inches, discs could be made without external evidence of porosity.

When direct acrylic filling materials are polymerized in such a mold, the discs which result appear to be firmly adhering to the sides of the mold for it is always necessary to hammer them from the mold (with a leather or rubber mallet). The top or bottom flat surfaces are the areas where almost all of the shrinkage occurs and the extent of this shrinkage depends on the type of surface against which the material is cured.

It was hoped that if the shrinkage, on the top surface, for example, was uniform so that a regular spherical surface would be formed, it would be possible to survey the surface with a dial-gauge and calculate the volume of the concavity. The surfaces of numerous discs were surveyed in this manner but in all cases the surfaces were irregular and it was considered that the calculation of volumetric shrinkages from the data obtained would be most inaccurate.

Volumetric shrinkages as presented in this report were determined by the displacement of a liquid. In some cases distilled water was used and ethylene glycol was also found very satisfactory because of its good wetting properties and slight tendency toward bubble formation.



## Experimental Procedure

Kadon, Fastcrown and Replica brands of direct acrylic filling materials were investigated. The ratios of powder to liquid used were one gram: one cc. for Kadon and Replica and 1.3 grams: one cc. for Fastcrown. The consistencies of the mixes for Replica and Fastcrown were possibly thinner than would be used in practice. The corresponding values for volumetric shrinkage would therefore tend to be slightly higher than would be encountered in actual use.

After measuring the powder and liquid and mixing them, the material was packed into the steel mold, compressed by a glass plate to squeeze out the excess resin and clamped under pressure for one hour. At the end of this time, the cured discs were removed, weighed in air and then weighed in ethylene glycol to determine their volume. The original mold volume was determined by reassembling the mold, leaving any existing flash in place, filling the mold with mercury in the same way as the resin was packed and then weighing the mercury. The discs were stored in air at room temperature and weighed again after 24 hours.

Three types of surfaces were used in this work. The lower surfaces of the resin discs were processed against the polished steel of the mold itself or against a thin sheet of polyethylene which was placed over the steel bottom. The upper surfaces of the discs were cured against glass or polyethylene. Because of technical difficulties the circumferential walls of the mold were not treated to make them non-adherent and were used in the condition which was imparted to them by machining operations.

The glass and steel provide surfaces to which the filling materials adhere when dry to a greater or lesser extent, depending on the resin. Since polyethylene is insoluble in nearly all solvents, it provides a non-adherent surface.

## Results

Below in tabular form are listed the volumetric shrinkages of Kadon, Fastcrown and Replica as determined in a closed mold against various surfaces. The figures are averages of three determinations made for each set of conditions.

|           | VOLUMETRIC SHRINKAGES WHEN CURED AGAINST |                 |                           |                 |              |                |
|-----------|------------------------------------------|-----------------|---------------------------|-----------------|--------------|----------------|
|           | Glass - Polyethylene                     |                 | Polyethylene-Polyethylene |                 | Glass-Steel  |                |
|           | <u>1 hour</u>                            | <u>24 hours</u> | <u>1 hour</u>             | <u>24 hours</u> | <u>1 hr.</u> | <u>24 hrs.</u> |
| Kadon     | 8.1                                      | 8.6             | 8.1                       | 8.2             | 5.6          | 6.2            |
| Replica   | 8.2                                      | 8.8             | 8.8                       | 9.0             | 1.0          | 1.5            |
| Fastcrown | 7.9                                      | 8.0             | 7.8                       | 8.3             | 0.3          | 0.8            |



Comments

The values given above for volumetric shrinkages are in fair agreement with values for free volumetric shrinkages determined dilatometrically. For example, dilatometer readings give for Kadon 7.4%, Fastcrown 6.5% and Replica 7.8%.

The differences may be attributed to experimental difficulties in determining the true original volumes of the discs and of the samples placed in the dilatometer. For example, when the discs are molded there is always produced a thin flash of resin, the thickness of which may vary slightly from one sample to another. The flash may alter the true mold volume by 1 to 2 per cent and it is not always possible to obtain a correct value of the original volume by reassembling the mold. When making dilatometer measurements, readings cannot be taken until the sample is workable enough to be inserted and until the apparatus is assembled and balanced. This may require three or four minutes and during this time some shrinkage occurs. Results must then be extrapolated to zero time to determine the original volume. Furthermore, the discs are more likely to be denser because they are cured under pressure while dilatometer samples, although condensed as much as possible before use, are not under pressure after measurements have begun.

Of special importance in connection with the results given in the above table are the locations of the shrinkages. In the glass-polyethylene cases, the discs were observed to adhere to the glass and smooth mirror-like surfaces were produced. The adhesion could be broken by inserting a wedge and allowing a thin film of water to penetrate between the glass and the resin. The surfaces next to the polyethylene were irregularly depressed indicating that shrinkage had occurred here. Since the circumferential walls of the steel mold were used in their natural state and filling materials adhered to these surfaces, the discs were always tightly gripped to the mold and had to be hammered out. Measurements of the diameters of the mold and of the discs revealed that volumetric shrinkages in this direction were not greater than about 0.3 per cent maximum.

The discs which were processed against polyethylene on both sides showed shrinkages on both surfaces and, as the table indicates, the total shrinkage was the same within the limits of experimental error.

In the glass-steel cases, shrinkages, as measured, were considerably lower in all cases. Internal shrinkage occurred in all these discs, however, with the development of extensive porosity. Fastcrown showed the lowest volumetric shrinkage under these conditions and this can, no doubt, be explained by the greater adhesion, particularly to the steel, which was observed for this material. Considerable energy had to be exerted to release Fastcrown discs



from the steel bottom of the mold and, in fact, a few small pieces which covered internal pores close to the steel surface actually were torn from the discs and remained adhering to the metal. Again, Replica showed stronger adhesion to the steel than did Kadon and this is evident on examination of the shrinkage values.

The table also shows that all of the materials continued to shrink by a small amount for at least 24 hours. The additional decrease in volume is smaller than the expansion which, for denture base acrylics, is reported to occur in the same period because of water absorption.

There are three important aspects of these results which may be of special significance in clinical practice where direct acrylic fillings are placed in teeth. First, practically all of the volumetric shrinkage should occur on the free surface of the filling next to the matrix assuming, of course, that the filling material adheres to the cavity either by mechanical or chemical adhesion. It is important that whatever matrix material is used should not adhere to the filling material, or at least, that the adhesion of the filling to the tooth is greater than the adhesion to the matrix. If the matrix sticks to the resin, then internal porosity may be expected to develop in the filling.

If shrinkage occurs on the free surface of the filling then a depression or cavity may result if the matrix is made to conform tightly to the contour of the tooth. Where possible an excess of resin should be used in the cavity and then ground and polished to shape.

Finally, in view of the fact that adhesion has been observed to be strongest against dry surfaces and that a film of water between resin and glass can completely destroy the adhesion, it should be sound practice to prepare tooth cavities under the driest possible conditions.

### Summary

Volumetric shrinkages in steel molds have been determined for Kadon, Replica and Fastcrown brands of acrylic filling materials.

For specimens cured against glass on one side and polyethylene on the other, practically all of the observed shrinkage appeared to be located next to the polyethylene or non-adherent surface.

For specimens cast against polyethylene on both sides, shrinkage occurred on both surfaces.

For specimens cast against glass on one side and polished steel on the other less shrinkage occurred than in either of the above cases internal porosity increased as the volumetric shrinkage decreased.



The data show that the three brands differ in adhesive properties. Fastcrown, in particular, adhered very strongly to the steel mold.

Three important considerations of significance in the clinical use of direct acrylic filling materials have been pointed out. These factors are all fundamentally based on adhesion.

### Future Work

It is proposed to make some investigations in connection with adhesion of acrylic fillings to materials approximating natural teeth. In view of some clinical evidence that marginal leakage occurs when acrylic fillings are used some consideration will be given to methods which might improve adhesive properties and decrease volumetric shrinkage.

### Volumetric Shrinkage

It was pointed out earlier that the commercial brands of direct acrylic filling materials have unusually high volumetric shrinkages. Values of approximately 5 to 8% as measured here by a dilatometric method would not seem to be desirable physical properties of a dental filling. In a series of experiments where polymerization was carried out in a confined space such as is the case in a tooth cavity it was shown that the nature of the surface to which the filling material was cured had considerable effect in determining the location of the shrinkage.

D. R. Christie

November 27, 1950.

If, for example, the resin was cured between surfaces to which it adhered, such as glass and steel, almost all the shrinkage occurred internally and the resin was literally pulled apart with the formation of extensive porosity. When polymerization was effected against one adherent and one non-adherent surface, such as polyethylene, practically all of the shrinkage occurred next to that non-adherent or free surface. And finally, with two non-adherent surfaces the polymerization shrinkage was distributed about equally adjacent to each of these free surfaces. A further important observation was that in most cases the adhesion was destroyed by allowing water to creep between the resin and the surface to which it was adhering.

The significance of these results to the practical use of direct acrylic filling materials is as follows. When a direct acrylic filling is placed in a tooth cavity practically all of the volumetric shrinkage should occur next to the matrix, assuming of course, that there is some adhesion between the resin and the tooth. It is also important that the adhesion between matrix and resin be insignificant, otherwise internal porosity may be produced in the filling. Since adhesion seems to be greatest to dry surfaces, it is desirable that cavities be prepared under the driest conditions possible, perhaps even with slight desiccation of the cavity walls with alcohol before insertion of the resin.





## APPENDIX "A"

ONTARIO RESEARCH FOUNDATION  
Department of Chemistry

Dental Materials Research Fellowship

Report No. 5101 - DR 25

### Scope of this Report

This report describes some recent work on the adhesion of direct acrylic filling materials and summarizes the related matter of volumetric shrinkage which was presented previously.

### Volumetric Shrinkage

It was pointed out earlier that the commercial brands of direct acrylic filling materials have unusually high volumetric shrinkages. Values of approximately 5 to 8% as measured here by a dilatometric method would not seem to be desirable physical properties of a dental filling. Later, in a series of experiments where polymerization was carried out in a confined space such as is the case in a tooth cavity it was shown that the nature of the surfaces against which the filling material was cured had considerable effect in determining the location of the shrinkage.

If, for example, the resin was cured between surfaces to which it adhered, such as glass and steel, almost all the shrinkage occurred internally and the resin was literally pulled apart with the formation of extensive porosity. When polymerization was effected against one adherent and one non-adherent surface, such as polyethylene, practically all of the shrinkage occurred next to that non-adherent or free surface. And finally, with two non-adherent surfaces the polymerization shrinkage was distributed about equally adjacent to each of these free surfaces. A further important observation was that in most cases the adhesion was destroyed by allowing water to creep between the resin and the surface to which it was adhering.

The significance of these results to the practical use of direct acrylic filling materials is as follows. When a direct acrylic filling is placed in a tooth cavity practically all of the volumetric shrinkage should occur next to the matrix, assuming of course, that there is some adhesion between the resin and the tooth. It is also important that the adhesion between matrix and resin be insignificant, otherwise internal porosity may be produced in the filling. Since adhesion seems to be greatest to dry surfaces, it is desirable that cavities be prepared under the driest conditions possible, perhaps even with slight dessication of the cavity walls with alcohol before insertion of the resin.





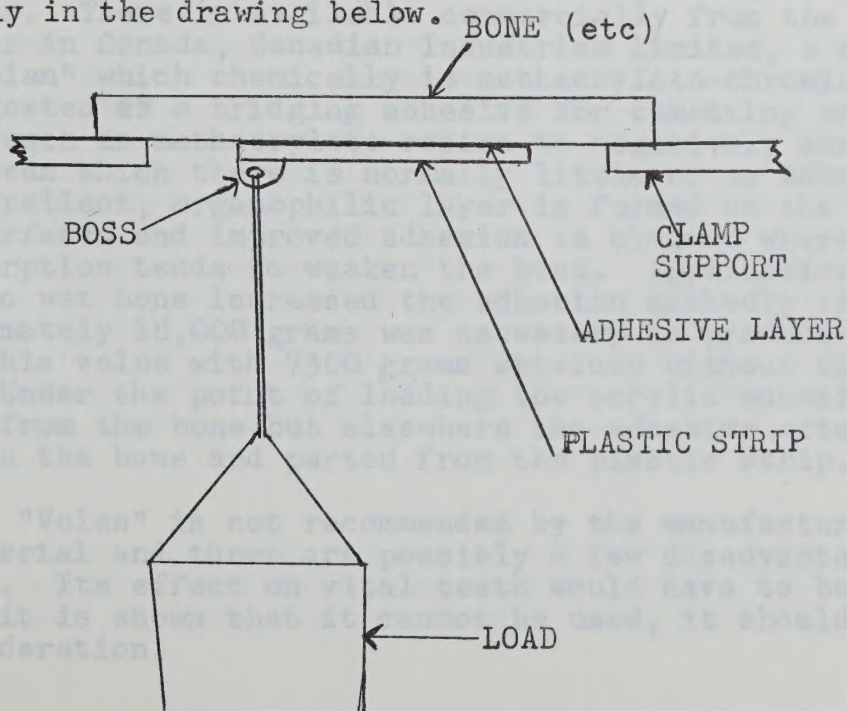


## Adhesion

It will be noted that in the argument above the assumption was made that some adhesion existed between the tooth and the acrylic filling material. To determine if there was any basis for making this assumption experiments have been made recently in which some relative values of adhesion of Kadon acrylic filling material to various surfaces were obtained.

The adhesion tests were made against glass, polished steel, polished stainless steel and to wet and dry bone. Bone was chosen as the material which probably would most closely approximate natural tooth dentine. For this purpose, beef rib bones were used because of their size and relative flatness. The bones were scraped clean, suitable areas were ground with a carborundum wheel to provide a rough surface and then rubbed with acetone to remove any traces of fat or grease. The bones were then conditioned in a dessicator at approximately 80% relative humidity for several days before use. In the dry bone adhesion tests the conditioned bones were wiped with absolute alcohol to remove any moisture. In the wet-bone experiments, the bone was soaked in water at room temperature for at least 24 hours, then wiped dry with absorbent cotton immediately before use.

The tests were carried out by cementing strips of polymerized Kadon measuring approximately  $1/8 \times 1/4 \times 1\ 1/2$  inches to the various surfaces using as the adhesive Kadon direct acrylic filling material. The strips were held in place under pressure for one hour. A boss of Kadon was attached at one end of the strip and through a hole in this boss a strong wire was passed to support the loading platform. The bone or other material was supported in clamps and a load of lead shot was added at a constant rate until the specimen failed. The experimental arrangement is shown diagrammatically in the drawing below.







"A-3"  
(Report No. 5101)

The results of these tests showed that the adhesion of Kadon acrylic filling material varies with the material against which it is cured.

The adhesion to glass was poor. The test specimens failed to withstand the initial weight of the platform which was less than 2000 grams.

Adhesion to polished stainless steel was appreciably stronger. The average load at failure for three specimens was 4500 grams. In fact, the magnitude of this adhesion at once raises the question as to whether stainless steel is a suitable matrix material for use in the placement of fillings in practice. Further research could be made along these lines.

In the case of bone which was soaked in water and wiped dry before the specimen was cemented to it, an average load of 7300 grams was required to break the adhesion. It is interesting to note that at failure the specimens separated completely from the bone.

The adhesion to dry bone was still greater and an average load of 12,000 grams was required to cause failure. In these tests, the specimen usually snapped in two near the point of loading leaving most of the strip adhering to the bone. The effect of a film of water on the adhesion is well demonstrated by a comparison of the values of the wet and dry experiments.

The strongest adhesion was found in the case of Kadon to polished steel where a load in excess of 14,000 grams was required to cause failure of the test pieces.

One other experiment should be mentioned as a matter of record in the event that future research may be carried out along these lines. There is available commercially from the DuPont Company, or in Canada, Canadian Industries Limited, a material called "Volan" which chemically is methacrylato-chromic chloride. It is suggested as a bridging adhesive for cementing organic materials such as methacrylate resins to negatively charged surfaces between which there is normally little or no adhesion. A water-repellent, organophilic layer is formed on the negatively charged surfaces and improved adhesion is claimed where normally water absorption tends to weaken the bond. Application of this material to wet bone increased the adhesion markedly and a load of approximately 18,000 grams was necessary to produce failure. (Compare this value with 7300 grams obtained without the use of "Volan") Under the point of loading the acrylic adhesive separated from the bone but elsewhere the adhesive actually remained on the bone and parted from the plastic strip.

"Volan" is not recommended by the manufacturers as a dental material and there are possibly a few disadvantages connected with it. Its effect on vital teeth would have to be established but until it is shown that it cannot be used, it should be given some consideration.







### Discussion of Adhesion Results

It was shown earlier that when direct acrylic filling materials were polymerized between two adherent surfaces, one of which was glass, sufficient adhesion existed between the glass and the resin to prevent surface shrinkage but to cause internal shrinkage or porosity. These present tests have demonstrated that adhesion to bone is considerably greater than adhesion to glass. And since bone and tooth dentine would no doubt adhere to acrylic fillings to about the same order of magnitude, it may be concluded that volumetric shrinkage of acrylic resins in tooth cavities can be controlled or localized to the free surfaces.

During the first hour, when direct acrylics undergo most of their shrinkage, it is important that real chemical adhesion exists between the tooth and the filling. The adhesion tests indicate that this is probably the case. Under such conditions the shrinkage will be confined to the free surface, that is, the area adjacent to the matrix. This supposes, of course, that the matrix material does not adhere in any way to the filling. Our results suggest that the suitability of stainless steel as a matrix material is open to question. An investigation of the effect of matrices on the shrinkage and internal structure of acrylic fillings might reveal valuable information. Suitable matrix materials would include inert resins such as polyethylene, probably the "Resinform" strips for use with Kadon and cellophane.

If a suitable matrix is used then the shrinkage of the free surface of a filling should produce a slight concavity. For this reason and where the contour of the filling is important, an excess of resin should be formed in the cavity, if possible, then ground and polished to the shape or contour of the tooth.

It has also been shown that the moisture content of the bone surface and, therefore, presumably, the surface of the tooth cavity affects the adhesion of the filling to the dentine. It is suggested that cavities be prepared under absolutely dry conditions and that the surfaces be dehydrated with absolute alcohol immediately before insertion of the filling. Although chemical adhesion of the acrylic filling to the tooth may be reduced or destroyed when the water content of the cavity walls returns to normal, the important factor of adhesion during polymerization will have existed and marginal adaptation will have been maintained as much as possible. In all probability mechanical adhesion will have to be relied on afterwards to hold the filling in place as, indeed, it does with most filling materials.

### Summary

Adhesion tests and studies of volumetric shrinkage in confined spaces have indicated that direct acrylic filling materials adhere to bone, and presumably tooth dentine, during







"A-5"  
(Report No. 5101)

polymerization and that shrinkage should be confined to the free surface of the filling.

The use of a non-adherent matrix is necessary to ensure marginal adaptation and freedom from porosity in acrylic fillings.

The presence of moisture on bone, and presumably tooth cavities, weakens the adhesion of direct acrylic fillings. Cavities should be prepared dry, kept dry and possibly even surface-dehydrated for best results.

Project No. B2 32

Amount of Grant: \$500.

SUMMARY D. R. Christie

Progress Report:

January 23, 1951.

Since cortisone administered to individuals with rheumatoid arthritis and rheumatic fever suppresses inflammatory and degenerative changes in the connective tissues of the joints and probably in heart valves also, all of which structures consist chiefly of collagen, it was believed worth while to investigate the state of growing connective tissues in animals, particularly bones and teeth, in cortisone deficient animals. Accordingly in a preliminary experiment the adrenal glands were removed from several growing rats and the rats were given D.C.A. Under these conditions they thereafter were supplied only with the mineral affecting cortical hormones and were deficient in the cortical hormones that affect protein and carbohydrate metabolism including cortisone.

To our surprise, histological sections of the growing bones of these animals showed no changes from the normal. The possibility of the rat perhaps being able to convert one cortical hormone to another was considered and it was decided to repeat the experiments with guinea pigs.

Although adrenalectomy is a very difficult operation in the guinea pig several growing guinea pigs, with the assistance of Dr. Bryden Smith, have now been adrenalectomized, given D.C.A. and killed at suitable intervals. Their bones and teeth are being sectioned at present. In addition, Dr. Bryden is now adrenalectomizing another series of young rats to study their teeth under conditions of deprivation of the different cortical hormones and in addition will study another series given additional cortisone.

This is just a progress report, a complete report will be filed for the Autumn meeting.

Date: 27 January, 1951.

"Arthur W. Hae"  
Signature





APPENDIX "B"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951

Subject: "Adrenal Cortical Hormones and the Growth of Bones and Teeth"

Grantee: Arthur W. Ham, M.B.

Project No. DR 32

Amount of Grant: \$900.

SUMMARY OF WORK

Progress Report:

Since cortisone administered to individuals with rheumatoid arthritis and rheumatic fever suppresses inflammatory and degenerative changes in the connective tissues of the joints and probably in heart valves also, all of which structures consist chiefly of collagen, it was believed worth while to investigate the state of growing connective tissues in animals, particularly bones and teeth, in cortisone deficient animals. Accordingly in a preliminary experiment the adrenal glands were removed from several growing rats and the rats were given D.C.A. Under these conditions they therefor were supplied only with the mineral affecting cortical hormones and were deficient in the cortical hormones that affect protein and carbohydrate metabolism including cortisone.

To our surprise, histological sections of the growing bones of these animals showed no changes from the normal. The possibility of the rat perhaps being able to convert one cortical hormone to another was considered and it was decided to repeat the experiments with guinea pigs.

Although adrenalectomy is a very difficult operation in the guinea pig several growing guinea pigs, with the assistance of Dr. Bryden Smith, have now been adrenalectomized, given D.C.A. and killed at suitable intervals. Their bones and teeth are being sectioned at present. In addition, Dr. Breen is now adrenalectomizing another series of young rats to study their teeth under conditions of deprivation of the different cortical hormones and in addition will study another series given additional cortisone.

This is just a progress report, a complete report will be filed for the Autumn meeting.

Date: 27 January, 1951.

"Arthur W. Ham "

Signature





APPENDIX "C"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951

Subject: Cements for methacrylate inlays

Grantee: Dr. A.E.R. Westman

Project No. DR 12      Amount of Grant: (\$3,000 includes DR 25)

SUMMARY OF WORK

Since direct acrylic filling materials have virtually replaced the acrylic inlay, most of our attention has been directed to the former materials which were authorized for investigation under project DR 25.

It is possible that in some instances a cement for an acrylic inlay would be an essential item. The results of our most recent work on project DR 25 have indicated that direct acrylic filling materials used as inlay cements would be more satisfactory than zinc phosphate cements.

The direct acrylics are available in many shades and being chemically the same as the inlay no aesthetic problems should be encountered. Adhesion of the direct acrylics to tooth structure is better than the adhesion of zinc phosphate cements which have been used in the past. There is the possibility that a thin layer of cement would require a somewhat lengthy period to polymerize. The application of a moderate amount of heat and the use of one of the more rapid-setting direct acrylics would likely overcome this problem. Further research would have to be done on this point, however.

The matter of polymer particle size would also have to be considered in more detail although it seems that a very fine polymer powder such as Kadon would be most suitable.

A.E.R. Westman

Signature

Date: 31 Jan. 1951.





## APPENDIX "D"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Experimental Fusospirochetal Infection with Mixtures of Pure Cultures

Grantee: John B. Macdonald

Project No. DR 35

Amount of Grant: \$2,000.

#### SUMMARY OF WORK

Attempts to subculture from 65 different colonies on various agar media inoculated with HG guinea pig exudate or HG mixed culture emulsions have yielded 20 pure cultures of organisms. These can be classified as streptococci (8 strains) non motile gram positive rods (6 strains) Bacteroides (3 strains) vibrio (1 strain) and spirochetes (2 strains). All strains are anaerobic except one streptococcus which is facultative.

Other morphologic forms can be seen in the exudate and efforts are therefore being directed at improving methods with a view to increasing isolations from HG. For this purpose 14 strains of anaerobic motile rods obtained from various sources is being used since this represents the group most refractory to efforts at pure culture. Experiments to improve methods include testing of a number of basal media, testing of various enrichment ingredients, and bacteriostasis with added dyes.

Three guinea pig exudates and three gingival scrapings were cultured on boiled sheep blood agar incubated either aerobically or in 10% CO<sub>2</sub> to test for the presence of pleuropneumonia organisms in these sources. No typical colonies were found but tiny colonies were examined by darkfield or Giemsa-stained impression smears. No pleuropneumonia forms were found.

Date: Feb. 7, 1951.

"John B. Macdonald"

Signature

#### Bacteroides

Three strains of gram negative non motile rods have been isolated. These may be Bacteroides although the first two strains were pleomorphic and showed gram positive granules. As with all





## APPENDIX "D"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Experimental Fusospirochetal Infection with Mixtures of Pure Cultures.

Grantee: John B. Macdonald

Project No. DR 35      Amount of Grant: \$2,000.

Work has continued on isolation of organisms from the "HG strain" of guinea pig fusospirochetal exudate using media and methods described in an earlier progress report. A total of sixty-five colonies have been subcultured. Organisms isolated to date can be classified in the following categories:

1. Streptococci
2. Non motile gram positive bacilli
3. Bacteroides
4. Vibrios
5. Spirochetes

#### Streptococci

Eight pure cultures of streptococci have been isolated, seven of which were anaerobic and one of which is facultative, producing non-hemolysing colonies on aerobic blood agar. Preliminary screening of these cultures to eliminate replicates has been limited to observation of colonial form, cell morphology Gram staining and ability to grow in 5 different fluid media. On this basis there appear to be at least 5 distinct strains.

#### Non motile gram positive rods

Six isolations of non motile gram positive rods have been made. Preliminary screening of this group has not suggested the presence of replicates although differences observed between strains may fall within normal strain variation which seems to be more extensive amongst anaerobes than aerobes. This group of organisms is characterized by pleomorphic cell morphology which is most evident in one strain--JR6. The latter appears as most bizarre forms -- twigs or branches or irregular bent rods with central or terminal swellings.

#### Bacteroides

Three strains of gram negative non motile rods have been isolated. These may be Bacteroides although the first two strains were pleomorphic and showed gram positive granules. As with all



organisms in this experiment, the names attached are provisional pending specific identification.

### Vibrios

A single culture of actively motile gram negative rods has been isolated from HG. It grew as flat translucent colonies on Proske Sayers blood agar and survived subculture in heart infusion--ascitic fluid--kidney extract medium. It appears in the fluid medium as tiny rods about 2 micra long, straight or slightly curved and exhibiting a very rapid darting back-and-forth motility.

### Spirochetes

Spirochetes have been isolated free from contaminating bacteria on two occasions. Since these organisms can not be grown as isolated colonies they can be considered pure only in the sense that they are free from bacteria. They may represent a mixture of different spirochetes although in both cases the cell morphology seems fairly uniform. They were isolated from a haze radiating from the edge of confluent growth of exudate on Proske Sayers blood agar. Agar from the haze was emulsified in a tissue grinder and inoculated into spirochete tubes. Abundant growth appeared in 6 days.

### Other forms seen in HG exudate

Examination of fresh guinea pig exudate or an emulsion prepared from mixed growth of the total flora discloses morphologic forms which have not been isolated in pure culture. These include particularly fusiform bacilli (which seem to be relatively few in number in this material though they are clearly present) and spirilla varying in length from 10 to 30 micra. These have been seen irregularly in culture emulsions and have never been numerous.

Evidence that there are organisms in the exudate which have not been cultivated makes it desirable to postpone recombination of the pure cultures until further isolations can be achieved either by improving the methods or by persevering with the present techniques. It is therefore planned to proceed along these lines during the next few months.

Since motile rods appear to be the most difficult of these organisms to cultivate, efforts to improve techniques are underway using these organisms for observations. Fourteen strains of motile anaerobic rods have been isolated from various sources and are now under study to determine their growth characteristics and requirements. (Eight of these were obtained from Dr. Theodor Rosebury and six were isolated in this laboratory. The total of 14 represents the largest number of strains of these organisms yet assembled.) The methods at present under investigation include the following:



1. Cross streaking of motile rods against a. yeast  
b. anaerobic cocci c. total exudate in an effort  
to demonstrate satellite formation. This would be  
evidence of growth factors emanating from the culture  
used for cross-streaking.
2. An evaluation of seven different base media for  
supporting growth of the 14 available strains. This  
will provide hitherto unavailable information concern-  
ing the choice of medium for subculturing and other  
purposes.
3. An assessment of various ingredients as growth stimulants  
when added to the choice basal medium or media as determined  
above. Ingredients tried will include yeast extract,  
casein hydrolysate, glass filtrate of guinea pig exudate  
and others.
4. Selective bacteriostasis experiments. An experiment  
is underway to observe the ability of organisms in  
mixed exudate to survive serial passage in liquid  
medium at three day intervals. To date exudate has  
been subcultured six times by this method. Dark field  
examination indicates considerable simplification of  
the flora by this technique. Cocci and motile rods  
have survived in large numbers, non motile rods in small  
numbers. Spirochetes and fusiforms have been eliminated.  
It is planned to try and eliminate or reduce the numbers  
of cocci by adding to the medium various concentrations  
of different dyes--gentian violet, brilliant green and  
methylene blue. If this should be successful it would  
permit inoculation of solid media with the likelihood  
of heavy growth of motile rods and little or no growth  
of other organisms. This technique has been used  
successfully in cultivating fusiform bacilli by Bøe (1941)

#### Pleuropneumonia organisms

-like

The presence of a pleuropneumonia/organism in primary  
fusospirochetal gangrene of the penis was recently reported  
by Ruiter and Wentholt (J. Invest. Derm. 15 = 301: 1950). This  
raised the question of the presence of such organisms as an  
essential component of fusospirochetal exudates. An experiment  
was performed to test for the presence of these organisms in  
various fusospirochetal materials. Boiled sheep blood plates  
were prepared according to the formula of Ruiter and Wentholt.  
Two plates were streaked from each of six sources--three guinea  
pig exudates (one of which was HG) and three suspensions of  
gingival scrapings (thawed from CO<sub>2</sub> ice storage). One plate  
from each source was incubated aerobically and one in an  
atmosphere of 10% CO<sub>2</sub>, at 37° for 5 days at which time the



plates were examined under the colony microscope at a magnification of 20X. Tiny translucent colonies were found in the zones of heavy inoculum but these were in no instance typical of the descriptions of pleuropneumonia colonies. Random samples were examined under darkfield and showed either cocci or rods. This experiment was repeated using only 2 strains of guinea pig exudate and making Giemsa-stained impression smears from the agar surfaces. The protoplasm of pleuropneumonia organisms has been described by Klieneberger as "attenuated" and is subject to easy distortion from handling--hence the impression slides. These slides also showed cocci only. It is concluded that in this material there are probably no pleuropneumonia organisms.

John B. Macdonald

Signature



## APPENDIX "E"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Chemical Mechanisms in Dental Caries

Grantee: Dr. J. J. Rae

Project No. DR 1 Amount of Grant: \$950

Period Covered: Feb. 1st, 1950 to Feb. 1st, 1951.

#### SUMMARY OF WORK

(1) A paper entitled "Ammonia Production in Saliva" has been accepted for publication in a forthcoming issue of the Journal of Dental Research. This work was performed in the main by Miss Ballantyne of the Consumers Research Laboratories and showed that there is present in saliva an ammonia producing mechanism which is inhibited by sodium fluoride (0.25%) and glucose (2.5%). Heating to 50° destroys the ammonia producing mechanism. The addition of urea produces 5-10 times as much ammonia showing the presence of an active urease in saliva. Glucose, strangely enough, stimulated ammonia production to twice its value over that observed when urea alone was added to saliva. The ammonia producing mechanism is probably bacterial in origin since it is removed by Seitz filtering the saliva. This work has been extended to include a larger number of individuals (50) and the results show that there is no apparent relationship between l.b.a. counts of salivas and ammonia production, or between l.b.a. counts and urease activity. Another paper on this subject is almost ready for submission to the Journal of Dental Research.

(2) A bactericidal substance for lacto bacillus acidophilus was prepared from ground tooth enamel by means of bromoform extraction and many attempts have been made to prepare it in pure form without success. Crystals prepared from the extract, although not identified, were found to have no bactericidal effect. The bactericidal material is adsorbed on charcoal from an alcoholic solution of the material. On evaporation of an alcoholic solution of the material the chloroform insoluble part of the residue was bactericidal and separation of this into two fractions by sodium hydroxide showed activity in both the precipitate and the solution.

(3) Three short-term projects were performed for members of the Dental Faculty. For Dr. C.H.M. Williams the pH of a series of carbonated beverages was determined and a possible explanation as to why the pH of such drinks drops on standing was advanced. For Dr. Cox a preliminary experiment on Vitamin B<sub>12</sub> showed that it had no marked effect on the growth of l.b.a. Also for Dr. Cox the effect of the end-products of metabolism of l.b.a., grown in the usual culture medium, on the growth of l.b.a. was investigated. No inhibitory effect was observed at the concentrations used.

J. J. Rae

Signature







## APPENDIX "E"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

#### ANNUAL REPORT

(Interim Report No.11)

Subject: Chemical Mechanisms in Dental Caries

Grantee: J.J. Rae

Project No. DR 1

Period Covered: Feb. 1, 1950 to Feb. 1, 1951.

The work covered in the period Feb. 1, 1950 to Sept. 15, 1950 was dealt with in detail in Interim Report No. 10 and at that time the work was subdivided into two main parts.

- (a) Ammonia Production in Saliva and
- (b) A Growth Inhibitor for *Lactobacillus Acidophilus* (l.b.a.) in Tooth Enamel.

In addition to these two major projects three minor problems have been undertaken.

- (c) The pH of Carbonated Beverages
- (d) The Effect of Vitamin B<sub>12</sub> on the Growth of l.b.a.
- (e) The Effect of the End-products of l.b.a. Growth in the Growth of l.b.a.

#### (a) Ammonia Production in Saliva

The work covered - February 15th to Sept. 15th, 1950 was embodied in Section A of Interim Report No. 10, and was submitted to the Journal of Dental Research for publication. We have just heard that this paper has been accepted for publication in the near future.

During the fall, Miss Ballantyne working at Consumers Research Company laboratories has been able to extend the work outlined in this paper to a much larger number of salivas (about 50) on which the following determinations have been made:

- (a) L.b.a. counts (Miss Berry at Dental College).
- (b) mg% NH<sub>3</sub> produced in 24 hours
- (c) mg% NH<sub>3</sub> produced after addition of urea
- (d) mg% NH<sub>3</sub> produced after addition of urea and glucose.



The results showed that the addition of glucose doubled the production of  $\text{NH}_3$  from saliva and urea. This stimulating effect of glucose in urease is to be investigated further.

No co-relation was found between l.b.a. counts of saliva and ammonia production in 24 hours; or between l.b.a. count and urease activity of saliva.

It is intended to submit the results of this investigation for publication to the Journal of Dental Research in the near future.

(b) A Substance from Tooth Enamel which has a Bactericidal Action of Lactobacillus Acidophilus

In Section B of Interim Report No. 10 (Sept. 15th, 1950) it was shown that when tooth enamel was shaken with bromoform to separate the dentine and enamel (Manly-Hodge method), the dried enamel exhibited a bactericidal action on l.b.a. The addition of  $\text{Ca}_3(\text{PO}_4)_2$  had no deleterious effect on the growth of l.b.a., showing that the bactericidal effect observed was not due to buffer action, calcium-ions or phosphate-ions.

Bromoform itself had no bactericidal effect on l.b.a. It was further shown that extracting the dried enamel with 95% ethanol produced an extract which had bactericidal properties. Ether extracts also had a bactericidal effect. Qualitative tests on the alcoholic extract of dried bromoform-treated enamel indicated the absence of sulphur, nitrogen and phosphorus but indicated the presence of halogen ion. The alcoholic extract contained calcium whereas the ether extract contained only a trace of calcium.

Since the inhibitory substance is not calcium phosphate and is soluble in organic solvents, it is possible that the organic fraction of whole teeth may contain the inhibitory substance.

Whole teeth were decalcified by immersion in 5% nitric acid for 14 days. The organic residue from this treatment, when shaken with bromoform, separated and dried, showed no inhibition of growth of lactobacillus.

The following procedure was evolved for the extraction of the bactericidal substance.

Ground tooth enamel is shaken with 4 volumes of bromoform for 15 minutes. The mixture is then filtered and the residue is air dried at  $160^\circ$  for 3 hours to remove bromoform. The dried residue is then extracted in a Soxhlet for 4 hours. The alcohol extract had a bactericidal effect on l.b.a.

Subsequent work (Sept. 15th to Feb. 1st) on the alcoholic extract prepared as above has shown:



(1) That, when evaporated to a small volume, a creamy white precipitate forms. When the precipitate was separated by filtering, dissolved in chloroform, and evaporated, crystals resulted. After washing with chloroform and recrystallizing, a micro-analysis was performed. The crystals contained 4.48% carbon and 0.79% hydrogen and had a sharp m.p. of 114.5°. These crystals were tested against l.b.a. and we found that the crystals did not inhibit l.b.a. growth.

(2) That, when the alcoholic extract was shaken with charcoal to remove the yellow colour, the bactericidal substance was also removed by adsorption on the charcoal. Tenth normal sodium hydroxide and tenth normal hydrochloric acid, when shaken with the charcoal, did not remove the bactericidal substance.

(3) That, when the alcoholic extract is evaporated to dryness, the residue can be divided by chloroform into chloroform soluble and chloroform insoluble portions. The chloroform insoluble part was dissolved in 10 ml. of 95% ethanol and 0.2 ml. of saturated alcoholic solution of sodium hydroxide was added. A white precipitate formed in 1 hour. On centrifuging and testing against l.b.a. both the centrifugate and the precipitate were found to contain the bactericidal substance.

The chloroform soluble part on evaporation, solution in alcohol and the addition of sodium hydroxide gave a dark brown precipitate after standing for 4 days. After centrifuging, neither the centrifugate nor the precipitate had an inhibitory effect on l.b.a.

### (c) pH of Carbonated Beverages

At the request of Dr. C.H.M. Williams of the Dental College the pH of a group of carbonated beverages was determined by means of the Beckman pH meter. The results (pH) obtained were:

|                       |      |                    |      |
|-----------------------|------|--------------------|------|
| Wilsons Cola          | 2.52 | Nu Grape           | 3.12 |
| Wilsons Ginger Ale    | 2.62 | Wilsons Cream Soda | 3.20 |
| Coca Cola             | 2.66 | Seven-up           | 3.38 |
| Canada Dry Ginger Ale | 3.03 | Hires              | 4.15 |
| Wilsons Orange        | 3.05 |                    |      |

The titratable acidity of Coca Cola and Wilsons Cola was determined. 10 ml. of 0.1N sodium hydroxide was required to neutralize 25 ml. of the beverage.

Dr. Williams had reported that on standing the pH of Coca Cola dropped and in an attempt to answer this question a phosphoric acid solution (pH 2.88) was treated with CO<sub>2</sub> gas. The pH rose to 3.40 and on boiling to drive off CO<sub>2</sub> and cooling returned to pH 2.88. There is the possibility then that since phosphoric



acid is a stronger acid than carbonic acid the loss of CO<sub>2</sub> would lead to a drop in pH. This work has been suspended until a further request is received from Dr. Williams.

(d) Effect of Vitamin B<sub>12</sub> on Growth of Lactobacillus Acidophilus

On the suggestion of Dr. Cox of the Dental College, a few experiments were conducted in an attempt to show whether Vitamin B<sub>12</sub> had any effect on l.b.a. growth.

Squibb "Rubramin", 5cc. vials containing 30 micrograms per cc. were kindly supplied gratis by the Squibb Company. Tubes of broth culture medium + 0.2 ml. of the vitamin solution + 0.1 ml. of a diluted suspension of lactobacillus + varying amounts of glucose were incubated for 24 hours, then 0.1 ml. from each tube was plated on tomato agar medium. These plates were incubated for 4 days, then counted. Each plate showed a bacillus count of 1 million or more. No conclusion could be drawn from this result, since maximum growth occurred in all tubes.

Diluted broth culture, which, on testing contained 10,000 organisms per ml., was incubated for 24 hours in broth medium containing (a) no dextrose, (b) usual 1%. After plating and incubating for 4 days the plates gave counts as follows:

(a) no dextrose = 75,000

(b) 1% dextrose = 400,000

indicating that the dextrose was not needed for some growth of lactobacillus.

To complete this work an experiment should be done where the control tube shows little growth of lactobacillus. Then the stimulating or depressing effect of Vitamin B<sub>12</sub> could be noted.

(e) Effect of End-products of l.b.a. Growth on the Growth of l.b.a.

This was an attempt to answer the questions "Does Lactobacillus kill itself with the products of its own metabolism" and "If so, do these end-products have an inhibitory effect on the growth of a culture of lactobacillus?"

Eight ml. of broth culture medium was seeded with 6 colonies of lactobacillus picked with a loop from a tomato agar plate culture of lactobacillus and placed in an incubator at 37° for 35 days.

After 3 days the culture was tested by adding 0.1 ml. of culture to 4 ml. of broth culture medium, incubating 24 hours and plating 0.1 ml. on tomato agar plate material.



After 29 days 8 ml. of sterile distilled water was added to the culture tube making the total volume 10 ml., evaporation having taken place over the long period. 0.1 ml. was plated on tomato agar, incubated for 4 days and then counted.

Lactobacillus count = 1 million (3 days).

" = 0 (29 days).

The dead culture was filtered through a Seitz filter under reduced pressure. The filtrate was added in varying amounts to a 24 hour broth culture of lactobacillus.

The tubes were incubated for 24 hours and then 0.1 ml. was spread on tomato agar plates and incubated for 4 days. On removal from the incubator the colonies were counted expressing the results as number of lactobacillus in 1 ml. of broth culture.

|   | <u>Filtrate</u> | <u>Broth<br/>Medium</u> | <u>Count</u> |
|---|-----------------|-------------------------|--------------|
| 1 | 0.1 ml.         | 3.9 ml.                 | 480,000      |
| 2 | 0.3 ml.         | 3.7 ml.                 | 1 million    |
| 3 | 0.6 ml.         | 3.4 ml.                 | 1 million    |
| 4 | 1.0 ml.         | 3 ml.                   | 300,000      |
| 5 | none            | 4 ml.                   | 1 million    |

#### Conclusion:

There is no inhibition of the growth of lactobacillus, resulting from the soluble end-products of lactobacillus metabolism under these conditions.

CONCLUSIONS: These experiments indicate that the first premise upon which the investigation was based is valid, namely, that an anesthetized section of nerve may allow the passage of high frequency impulses although it blocks those of low frequency.

It is hoped that with the solution of the aforementioned technical difficulties, similar studies can be performed on sensory fibres which have been stimulated in a natural manner. In particular we hope to record directly the action potentials in the blocked inferior dental nerve when appropriate stimuli of various intensities are applied to the healthy and to the diseased teeth of the dog.





## APPENDIX "F"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951

Subject: A Study of Nerve Block

Grantees: Dr. L.B. Jaques and Mr. G.J. Millar

Project No. DR 31

Amount of Grant: \$1870

Purpose: A. To determine whether nerve impulses of high frequency will pass a section of nerve which is blocked to impulses of low frequency.

B. To determine the efficiency of blocking action of an anaesthetized section of the inferior dental nerve toward impulses arising from pain endings in healthy and hyperemic pulps.

Experimental Procedure: Work on this project has not progressed as favourably as was anticipated a year ago. The most of the delay has been brought about by failure to obtain adequate electric shielding for the cathode-ray oscilloscope with its high-gain preamplifier. The result has been that the investigation of primary interest with recordings from small sensory nerve fibres has not been accomplished.

Meanwhile a series of experiments with nerve-muscle preparations have been performed, the muscle serving as an indicator of nerve activity. The usual arrangement was to stimulate the nerve repetitively at a slow frequency (1 to 5 times per second), then apply anaesthetic to a segment of the nerve until partial or total block was produced as indicated by a diminished contraction or failure of contraction of the muscle. The frequency of stimulation was then increased.

RESULTS: It has been found that an enhanced rate of stimulation of the nerve will cause the muscle to contract although the nerve was blocked to the passage of impulses which were applied at a slow rate. The intensity of the applied stimulus was such that all nerve fibres were activated and the stimulator is so designed that variations in the output frequency and intensity are independent of each other.

CONCLUSIONS: These experiments indicate that the first premise upon which the investigation was based is valid, namely, that an anaesthetized section of nerve may allow the passage of high frequency impulses although it blocks those of low frequency.

It is hoped that with the solution of the aforementioned technical difficulties, similar studies can be performed on sensory fibres which have been stimulated in a natural manner. In particular we hope to record directly the action potentials in the blocked inferior dental nerve when appropriate stimuli of various intensities are applied to the healthy and to the diseased teeth of the dog.







## APPENDIX "G"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: A. An investigation of the Mechanism of Repair of the Supporting Structures of the Teeth  
B. Fusospirochetal Infection and Pre-existing Inflammation

Grantee: C. H. Best

Project No. DR 22

#### PART A

This work is being carried on in the Banting and Best Department of Medical Research by W. J. Linghorne, D.D.S., Research Associate, and D. C. O'Connell, M.S.A., Research Assistant, Banting and Best Department of Medical Research.

As reported to the Committee a considerable degree of soft tissue reattachment and regeneration of alveolar bone has been achieved under experimental conditions in dogs. So far the development of the experimental method in addition to providing a useful research tool has resulted in further knowledge of the potentiality of those tissues for repair and of the biologic mechanisms involved.

The findings to date indicate that there are two promising approaches for the development of a clinical procedure in the treatment of periodontal disease, i.e. a surgical approach and one that might be termed physiological. Both approaches are being investigated.

#### 2. Surgical Approach

Reattachment resulting from surgical intervention has been the claim of those advocating the so-called conservative approach to the treatment of periodontal disease. Whether or not reattachment has been achieved by these procedures is still controversial. Successful reattachment resulting from such treatment would depend on granulation tissue arising from the connective tissue of the gingiva having the potentiality to form cementum and bone in such an environment. This has never been established.

In the experimental studies resulting in reattachment and regeneration of the alveolar bone, the periosteum was utilized for the purpose of promoting repair by cells originating from the bony margins of the wound. However, it is not yet clear if the use of the periosteum resulted in this type of repair. The origin of the cells in the experimental reparative process is still in doubt. It appears to be essential that an answer must be provided to this question before a surgical clinical procedure can be developed.



Various trial studies are being carried out for the purposes of finding out a suitable approach to this problem. A brief discussion of some of the difficulties encountered in planning a suitable study might be of interest to the committee. A study of the repair of a lesion similar to that described in the experimental studies but with a gingival flap devoid of periosteum might give the answer. Such a lesion has been difficult to produce. The gingival flap in dogs is too thin to permit the surgical removal of the periosteum. Attempts to remove the periosteum by other methods have also been unsuccessful, resulting in the formation of a epithelialized pocket. Indirect methods for the production of the desired lesion are being carried out.

Various alternative methods for obtaining the desired information such as the use of colchicine, a drug that inhibits mitosis may be investigated if the more direct procedures described above are found to be unsuitable. Also consideration is being given to the use of other tissues such as the long bones or the spine. Obviously these approaches have certain disadvantages.

The problem of the epithelial lining of the pocket wall is the other great obstacle to the development of the clinical method. Indications so far suggest that this problem is also a difficult one. The outcome of the investigation for determining the origin of the required cells for the reparative process undoubtedly will have an important bearing on this problem.

### The Physiological Approach

So far this appears to be the most promising. Certain findings in the experimental studies suggest that the demonstrated ability of the periodontal structures to repair may have a place in the physiology of these tissues. Accordingly a study was planned to investigate this hypothesis. The results so far have been encouraging. However, further work is necessary before this project is ready for presentation to the committee.



PART B

This work was carried out in the Banting and Best Department of Medical Research, University of Toronto, by D.C. O'Connell, M.S.A., Research Assistant, Banting and Best Department of Medical Research.

Object: The object of this research is further knowledge of predisposing factors in fusospirochetal infections.

Introduction

A means of studying factors that may have a predisposing effect upon fusospirochetal infections is provided by experimental infections in laboratory animals. Experimental fusospirochetal infection in laboratory animals is produced by the subcutaneous inoculation of materials from disease processes which contains the fusospirochetal complex of organisms.

Such factors as diabetes deficiencies and products of inflammation are being investigated by means of the above technique.

A study of the infectivity of fusospirochetal exudates from disease processes has been made in guinea pigs, rats and rabbits in order to determine the various infecting doses so that an assay of the effect of predisposing factors can be made.

Gingival scrapings containing large numbers of fusospirochetal organisms injected into the subcutaneous tissues of guinea pigs give rise to sizeable necrotic abscesses which are filled with foul smelling exudate containing the fusospirochetal group or complex of organisms. These lesions are transmissible through guinea pigs and are routinely produced by the injection of known amounts of lesion exudate.

Procedure

Material such as gingival scrapings taken from disease processes is suspended in a small amount of suitable diluent. Darkfield examination is made to assure the presence of large numbers of fusospirochetal organisms. Usually 0.4 cc. of this mixture injected subcutaneously into guinea pigs will give rise to a sizeable necrotic abscess containing large numbers of anaerobic fusospirochetal organisms. After this experimental infection has been initiated, 0.1 cc. of the experimental lesion exudate on transfer by injection to other guinea pigs will give rise to uniform transmissible lesions. On this basis these amounts of infective materials have been used routinely in the development of experimental fusospirochetal infection.

Experimental

Routine storage and preservation of infective exudates is made possible by keeping the exudates in 1 or 2 cc. ampules in a dry-ice chest at a temperature of  $-70^{\circ}\text{C}$ . Such a chest has been constructed in this laboratory and very economically can preserve



a maximum of 720 ampules of material at one time. The design and construction of this chest will be included in a later report. A standard strain of fusospirochetal passage exudate of known quality was essential for use in proposed experiments. Such a strain was developed by initial infection of guinea pigs with material from a disease process and subsequent transfer of the infective exudate through other animals.

Exudates from nine disease processes were injected into guinea pigs. Infections were initiated with material from six of these cases and one was chosen and expanded into a standard strain of exudate by thirteen passages through guinea pigs. Transfer of the other infective exudates was terminated after from 1 to 5 passages.

Exudate from lesions in all animals in the series was examined by darkfield illumination. If the material met the microbiological requirements and if the lesion from which it was taken was typical, portions of the exudate was transferred to other guinea pigs and the remainder preserved for experimental use. In many cases the exudate was frozen for many days before transfer. Intervals of freezing did not seem to influence transmissibility of the standard strain.

A total of 133 guinea pigs were used in the development of standard strains of exudate and the one strain used in most of this work involved the use of 63 normal guinea pigs. Table I depicts the development of the standard strain of exudate. It can be seen in Table I that the time required for development of the experimental lesion in each animal, and the interval of freezing before passage have little bearing on subsequent animal infection. In some cases a longer time was allowed before tapping the abscesses for exudate so that a greater amount of infective material could be collected. Usually from 3 to 10 cc. of foul smelling exudate were collected from each animal in which a typical abscess developed. During the course of development of this strain only one animal failed to become infected. In a few cases the lesions ruptured and drained and consequently no exudate was collected from these animals. The standardization of exudate by guinea pig passage provided data on infectivity, transmissibility and preservation of the exudate in addition to a large quantity of infective material for use in other experiments.

Early in this investigation it was apparent that the use of the guinea pig in experiments carried out to determine predisposing effects upon fusospirochetal infections was limited. Except for the possibility of learning the effect that a deficiency of Vitamin C might have upon infectivity and susceptibility of the guinea pig to experimental infection with fusospirochetal exudates, no other deficiency studies could be planned because of the feeding habits of the guinea pig. Therefore a study of experimental fusospirochetal infection in the rat was carried out and a paper dealing with fusospirochetal infection in the rat, and the use of this information in deficiency studies in relation to fusospirochetal infection is now being prepared. Only the essential details of the study in rats will be reported at this time.



### Experimental Fusospirochetal Infection in Rats

1. White rats of the Wistar strain are susceptible to infection with exudates containing the fusospirochetal complex of organisms. They have been infected experimentally with material taken from disease processes in the mouth and also with exudate from experimental lesions in the guinea pig and the rat.
2. The white rat does not appear to be as susceptible to experimental fusospirochetal disease as is the guinea pig. The amount of inoculum required to effect a lesion of the same order in a rat as in the guinea pig, is greater by approximately one half than that amount required to give rise to a typical experimental lesion in the guinea pig.
3. The anatomical differences between the two species of animals are suggested as reasons why the responses to subcutaneous injections of fusospirochetal exudates are not very similar. The thin skin of the rat tends to rupture much more readily when an abscess is produced in the subcutaneous tissues. In addition there is a much greater tendency for the injected material to become localized and resorbed with smaller doses. The thinness of the skin of the rat and the tendency to localization with consequent rupture and drainage followed by very rapid healing minimizes death in rats due to infection with fusospirochetal exudates.
4. The susceptibility of the individual rat based on the size of lesion resulting from a specified dose must be determined within one week after inoculation.
5. From an experiment in which 80 normal rats received varying doses of a constant strain of fusospirochetal exudate it has been found that any amount less than 0.1 cc. injected subcutaneously will not consistently give rise to a typical lesion within one week.

### Experimental Fusospirochetal Infection in Rabbits

Previous workers have shown that normal rabbits are not very useful laboratory animals in investigations which necessitate the injection of fusospirochetal exudates beneath the skin. Subcutaneous injections of infective exudates tend to spread too rapidly beneath the skin of the rabbit and a confused picture of infection is the result. In addition, the chance contamination of the subcutaneous tissues with streptococci to which the rabbit is most susceptible tends to bring about early death of test animals.

For these reasons, intra-dermal injection of fusospirochetal exudate was attempted and found to be a suitable means of producing fusospirochetal lesions in normal rabbits.

Eight normal rabbits were injected in the shaved skin of the back with varying doses of a standard strain of fusospirochetal exudate. The doses injected were 0.05, 0.1 and 0.5 cc. diluted to 0.5 cc. Four injections of 0.05 cc; eight injections of 0.1 cc. and four injections of 0.5 cc. of exudate were made in these animals.



These lesions which developed were typical necrotic abscesses filled with thick foul pus which contained fusospirochetal organisms. The initial response observed hours after injection was one of erythema. This was followed by a swelling of the dermis and a spreading of the reaction beneath the upper layer of the skin. In some of the lesions there was an ulcerating centre at the sight of injection from which a clear serum exuded from 24 - 48 hours after inoculation. Darkfield examination of this material demonstrated fusospirochetal organisms. As time went on the abscesses enlarged, the centres became necrotic and dry. About the sixth day the larger abscesses ruptured and some exudate drained. Complete drainage never occurred in any abscess, but a coagulation of the material took place. On the ninth day after injection most of the animals were sacrificed and the lesions excised and preserved. Gross section of some of these showed as much as 2 cm. of thickening of the skin at the centre of the lesion area. The largest lesions were about 6 x 5 x 1.5 cm. in size. The smallest lesion was only 3 cm. in diameter and merely red and swollen.

The size of the lesions developed varied directly with the amount of inoculum injected. All of the lesions contained fusospirochetal exudate but the smaller ones tended to drain and dry up. One of the animals in which 0.1 cc. was injected subcutaneously by accident died on the 8th day and showed the great spread of exudate under the skin. A greenish discoloration of the skin about 12 x 10 cm. in area was seen at autopsy. Cause of death of the animal cannot be determined but only assumed to be due to toxic absorption. It is quite apparent from this preliminary experiment that the normal rabbit is quite susceptible to intradermal experimental fusospirochetal infection, and it is obvious that further experimental work must be carried out to determine a suitable infecting dose of fusospirochetal exudate to use in experiments with rabbits which have a bearing on predisposing effects such as diabetes brought about by the injection of Alloxan monohydrate.

### Study of factors that may have a predisposing effect upon fusospirochetal infections by means of experimental infection in laboratory animals

#### A. Diabetes

By the use of Alloxan monohydrate both rats and rabbits can be made diabetic. Experiments have been drawn up to determine if the diabetic state of an animal has any predisposing effect upon its susceptibility to experimental fusospirochetal infection. A preliminary experiment with diabetic and normal rats indicated that diabetic rats might be more susceptible to infection with fusospirochetal exudate than normal rats. Four diabetic rats which received 0.1 cc. of standard exudate developed necrotic lesions in six days which were comparable in size to lesions in normal rats injected with 0.2 cc. of the same material.

Outlined below are two experiments planned to ascertain the effect of diabetes on experimental fusospirochetal infection.



Experiment F. S. I. D. R. T.

Object To determine the infectivity of fusospirochetal exudates in rats treated with alloxan.

Method Test rats are to be made diabetic by subcutaneous injection of alloxan monohydrate (200 mg. per Kg. body weight). Crude glycosuria tests are to be carried out 24 and 48 hours after treatment with alloxan. Rats showing positive sugar urines will be ready for infectivity testing.

Inoculum Rat-passed fusospirochetal exudate. Three groups of 4 rats will be injected with test exudates in amounts of 0.01, 0.05 and 0.1 cc. subcutaneously in the inguinal region. Three control groups of animals will be similarly injected.

|             |                       |            |
|-------------|-----------------------|------------|
| Group 1 - 4 | alloxan diabetic rats | - 0.01 cc. |
| " 2 - 4     | " " "                 | - 0.05 "   |
| " 3 - 4     | " " "                 | - 0.1 "    |
| " 4 - 4     | normal control rats   | - 0.01 "   |
| " 5 - 4     | " " "                 | - 0.05 "   |
| " 6 - 4     | " " "                 | - 0.1 "    |

Normal guinea pigs will also be injected to serve as controls on the exudate used and for comparison of lesions in both species.

Experiment F. S. I. R. B.

Object To study infectivity of fusospirochetal exudates in rabbits treated with Alloxan.

Method Test rabbits of either sex are to be made diabetic by the intravenous injections of Alloxan (200 mg. per Kg. body weight). Crude glycosuria tests are to be carried out 48 hours after treatment with Alloxan. When the rabbits are known to be diabetic they will be ready for infectivity testing. At this time they will be divided into 3 groups of 4 rabbits each and injected intradermally with different amounts of exudate from abscesses in guinea pigs in which experimental Vincent's infection has been induced.

A control group of rabbits will be similarly treated.

|             |                    |                       |
|-------------|--------------------|-----------------------|
| Group 1 - 4 | rabbits to receive | 0.01 cc whole exudate |
| " 2 - 4     | " " "              | 0.02 " " "            |
| " 3 - 4     | " " "              | 0.05 " " "            |
| Control     |                    |                       |
| 4 - 4       | " " "              | 0.01 " " " ) Diluted  |
| 5 - 4       | " " "              | 0.02 " " " ) with     |
| 6 - 4       | " " "              | 0.05 " " " ) saline   |
|             |                    | ) to                  |
|             |                    | 0.1 cc.               |



## B. Deficiencies

### 1. Deficiency of Vitamin C

Guinea pigs maintained on a diet of Purina Rabbit Chow Checkers become scorbutic in about three weeks and show the symptoms of their deficiency very markedly.

To determine the effect of a deficiency of Vitamin C on experimental fusospirochetal infection, two groups of guinea pigs were made scorbutic. These animals were injected subcutaneously with a standard strain of fusospirochetal exudate but at two different times. The first group after three weeks on the deficient diet, and the second group after seven weeks. Normal control animals were injected at the same time with the same exudates.

#### Results

| <u>Group I</u> | <u>18 scorbutic guinea pigs</u> | <u>Negative</u> | <u>Positive</u>   |
|----------------|---------------------------------|-----------------|-------------------|
| 4              | received 0.01 cc exudate        | 4               | 0                 |
| 4              | " 0.05 " "                      | 0               | 4                 |
| 4              | " 0.1 " "                       | 0               | 4                 |
| 6              | normal guinea pigs              |                 |                   |
| 2              | received 0.01 cc exudate        | 2               | 0                 |
| 2              | " 0.05 " "                      | 0               | 2 minimal lesions |
| 2              | " 0.1 " "                       | 0               | 2                 |

6 uninjected scorbutic guinea pigs died of scurvy at intervals after the injected animals were sacrificed.

Group II Only seven animals in this group of 13 survived for seven weeks. Six died of scurvy between the 4th and 7th weeks.

#### Results

| <u>7 scorbutic guinea pigs</u> | <u>Negative</u>         | <u>Positive</u>   |
|--------------------------------|-------------------------|-------------------|
| 4 received 0.01 cc exudate     | 2                       | 2                 |
| 3 " 0.05 " "                   | 1 early scorbutic death | 2                 |
| 11 normal guinea pigs          |                         |                   |
| 4 received 0.01 cc exudate     | 4                       | 0                 |
| 3 " 0.05 " "                   | 1                       | 2 Minimal lesions |
| 4 " 0.1 " "                    | 0                       | 4                 |

It would appear from these two experiments that scorbutic guinea pigs are more susceptible to experimental fusospirochetal infection than are normal guinea pigs. However mildly scorbutic animals do not appear to be susceptible to very small doses of exudate, whereas, severe infection does occur in severely scorbutic



animals. Although the control animals developed only minimal lesions when injected with 0.05 cc. of exudate, one-half of the usual infecting dose, the scorbutic animals developed typical lesions. The usual dose of 0.1 cc. of exudate gave rise to typical lesions in both normal and scorbutic guinea pigs.

## 2. Deficiency of the B Vitamins and Choline

Outlined below is an experiment designed to determine the effect of a deficiency of the Vitamin B complex on experimental fusospirochetal infection. This experiment is now in progress and nearly all the deficient animals both infected and uninfected are dead. No comparison of lesions in the deficient and in the paired-fed and normal controls can be made because the animals in the last two groups have not been sacrificed as yet.

### Experiment OCA

Object To study infectivity of fusospirochetal exudates in rats deficient in B Vitamin complex.

Method Set out 117 rats (125-150 gms.) in individual cages and divide among 12 comparable groups as to weight and sex distribution as follows:

- |      |         |        |                                                                            |
|------|---------|--------|----------------------------------------------------------------------------|
| I.   | OCA-0-1 | 9 rats | Feed diet OCA-0 (B deficient) ad lib. to these four groups.                |
|      | -2      | 9 "    |                                                                            |
|      | -3      | 9 "    |                                                                            |
|      | -4      | 16 "   |                                                                            |
| II.  | OCA-1-1 | 9 "    | Feed diet OCA-1 (with B vitamins) paired-fed with those in first grouping. |
|      | -2      | 9 "    |                                                                            |
|      | -3      | 9 "    |                                                                            |
|      | -4      | 10 "   |                                                                            |
| III. | OCA-2-1 | 9 "    | Feed diet OCA-2 (with B vitamins) ad lib. to these four groups.            |
|      | -2      | 9 "    |                                                                            |
|      | -3      | 9 "    |                                                                            |
|      | -4      | 10 "   |                                                                            |

When the rats on OCA-0 are believed to be sufficiently deficient in B vitamins (probably in about 3 weeks) all in first three sub groups of each group are to be injected subcutaneously as follows:

- |      |         |                                    |              |
|------|---------|------------------------------------|--------------|
| I.   | OCA-0-1 | inject with 0.01 cc whole exudate) |              |
|      | -2      | " " 0.05 " " "                     |              |
|      | -3      | " " 0.1 " " "                      |              |
| II.  | OCA-1-1 | " " 0.01 " " "                     | Diluted with |
|      | -2      | " " 0.05 " " "                     | saline to    |
|      | -3      | " " 0.1 " " "                      | 0.1 cc.      |
| III. | OCA-2-1 | " " 0.01 " " "                     |              |
|      | -2      | " " 0.05 " " "                     |              |
|      | -3      | " " 0.1 " " "                      |              |



## "G-10"

- |      |         |      |                                                                                                         |
|------|---------|------|---------------------------------------------------------------------------------------------------------|
| I.   | OCA-0-4 | (16) | } Control animals not to be injected but allowed to live at least until all the injected rats are dead. |
| II.  | OCA-1-4 | (10) |                                                                                                         |
| III. | OCA-2-4 | (10) |                                                                                                         |

Feed groups I and III ad. lib.

Feed group II paired-fed with group I

Record daily food consumption

Weigh rats on 7, 14, 18 and 21 days.

### Diet OCA-0

|                 | %     |
|-----------------|-------|
| Gelatin         | 2     |
| Casein          | 12    |
| Fibrin          | 1     |
| Sucrose         | 71    |
| Beef fat        | 5     |
| Corn oil        | 2     |
| Salts           | 5     |
| P.D.W. vitamins | 0     |
| Cod liver oil   | 0.015 |
| tocopherol ac   | 0.010 |
| Choline Cl      | 0     |

### Diet OCA-1 (OCA-2)

|       | % |
|-------|---|
| same  |   |
| same  |   |
| "     |   |
| 68.65 |   |
| same  |   |
| same  |   |
| same  |   |
| 2.0   |   |
| same  |   |
| same  |   |
| 0.35  |   |

### Results to date

The majority of the deficient animals were dead within eight days of the injection of the first three subgroups. These include the dietary controls. Therefore death of the animals cannot be attributed to the infection but to the deficiency. As mentioned, earlier consideration is given to the size of the lesions developed in the rats in one week after injection. All of the deficient rats showed evidence of infection except two and these died from the deficiency soon after injection. The animals which received 0.01 cc. of exudate developed necrotic lesions which contained foul smelling fusospirochetal pus. These varied in size from 0.75 cm. to 2 cm. in diameter. The dosage of 0.05 cc. of exudate in the second subgroup initiated lesions about 3 cm. in diameter. The largest dose of 0.1 cc. produced large lesions in all animals.

Comparison of the results in the normal rats to be obtained later with those in the deficient rats already recorded will complete this experiment.

### C. Products of Inflammation

#### 1. Need of Trauma

Preliminary experiments in this laboratory have indicated that trauma by movement of the injection needle beneath the skin just



prior to injection is not necessary to initiate fusospirochetal lesions in normal guinea pigs and rats.

In one instance 6 guinea pigs and 8 rats were injected with fresh material from six cases of necrotic periodontitis. Each animal received 0.4 cc. of gingival scrapings in a small amount of broth. This was a heavy suspension of organisms and lesion debris. Trauma was applied to half of the animals before injection. After a suitable interval of time all of the animals were sacrificed. Autopsy revealed no difference in size of lesion or severity of reaction between the two groups of guinea pigs and the two groups of rats.

## 2. Exudate

Preliminary experiments with fractions of whole fusospirochetal exudates have indicated several things which merit further experimentation.

1. Whole exudate will infect laboratory animals.
2. The solids (organisms and debris) suspended in saline will infect animals.
3. Sterile exudate filtrates injected in small amounts give rise to only very slight tissue reaction.

It is technically difficult to cultivate all of the organisms contained in a fusospirochetal exudate, but improved methods have made such cultivation possible. Cultivation of the organisms has not been attempted in this laboratory. Experiments are planned to increase our knowledge of the fractions of fusospirochetal exudates. The following experiment is planned and will be carried out with the co-operation of other workers.

1. Whole exudate - 4 guinea pigs
2. Killed whole exudate - 4 guinea pigs
3. Killed whole exudate solids in saline - 4 guinea pigs
4. Killed whole exudate liquid fraction - 4 " "
5. Killed whole exudate solids added to washed solids (living) - 4 guinea pigs.
6. Killed whole exudate liquid fraction added to washed solids - 4 " "
7. Killed whole exudate solids added to culture mixture of organisms - 4 guinea pigs
8. Killed whole exudate liquid fraction added to culture mixture - 4 "
9. Culture mixture of organisms - 4 guinea pigs.

A study of the tissue reactions in animals injected with the above preparations may clarify the inflammatory phase of fusospirochetal infection.

## Conclusion

When the results of all experiments planned and completed are correlated, it is hoped that this research will contribute knowledge of factors which have a predisposing effect on fusospirochetal infection.







O-1 Guinea-pig

Frozen 8 days





## APPENDIX "H"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Development of a method for determining fluorine by means of a photo-electric colorimeter.

Grantee: Dr. O. J. Walker

Project No. DR 28

Amount of Grant: \$1,000

#### SUMMARY OF WORK

(1) With the clearing up of some questionable points - a satisfactory photoelectric method for determining fluorides by the zirconyl salt - sodium alizarin sulfonate method has been developed. A paper on this part of the research is in preparation.

(2) A method for determining small amounts of phosphates has been developed and doubtful points in connection with certain variables have been checked. A paper embodying the results will be prepared for publication shortly.

(3) Conditions for determining small amounts of fluorides by the fading of the aluminum-hematoxylin lake are being established and the work in this phase is in an advanced state.

(4) Work is still to be done on determining somewhat larger quantities of fluorine and phosphate in such organic materials as bones, teeth, etc.

(5) Further studies on determination of fluorine in water, etc. are contemplated.

February 2, 1951

" O. J. Walker "

Signature





- 8 -

APPENDIX "H"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

February, 1951

Subject: Development of a method for determining fluorine by means of a photo-electric colorimeter.

Grantee: Dr. O.J. Walker

Project No.: DR 28

Amount of Grant: \$1000

This report is divided into five parts.

Part I

Determination of Small Amounts of Fluorides in a Photoelectric Colorimeter by the bleaching of the Lake Formed Between a Zirconyl Salt and Sodium Alizarin Sulfonate

Procedure

Reagents: These are the same as used in the Scott visual method (1).

Instruments: The absorption in the visual spectrum was determined by a Beckmann B Spectrophotometer. The measurements of fluoride were made in a 15 cm. absorption cell and a 515 m $\mu$  light filter.

It has previously been shown (see previous reports) that the method gives good results when certain specific directions are followed. There were, however, a number of variables that had not been studied closely enough. These are embodied in this report.

1. Effect of Using a New Indicator Solution

A series of standards was prepared containing 0.0 p.p.m. to 1.4 p.p.m. fluoride ion using two different lots of indicator. A new calibration curve must be prepared when a new lot of indicator is made up as shown by figure I. The table is shown below:

FIGURE I.

% TRANSMISSION VS. ppm F-  
SHOWING THE EFFECT OF DIFFERENT  
LOTS OF INDICATOR.

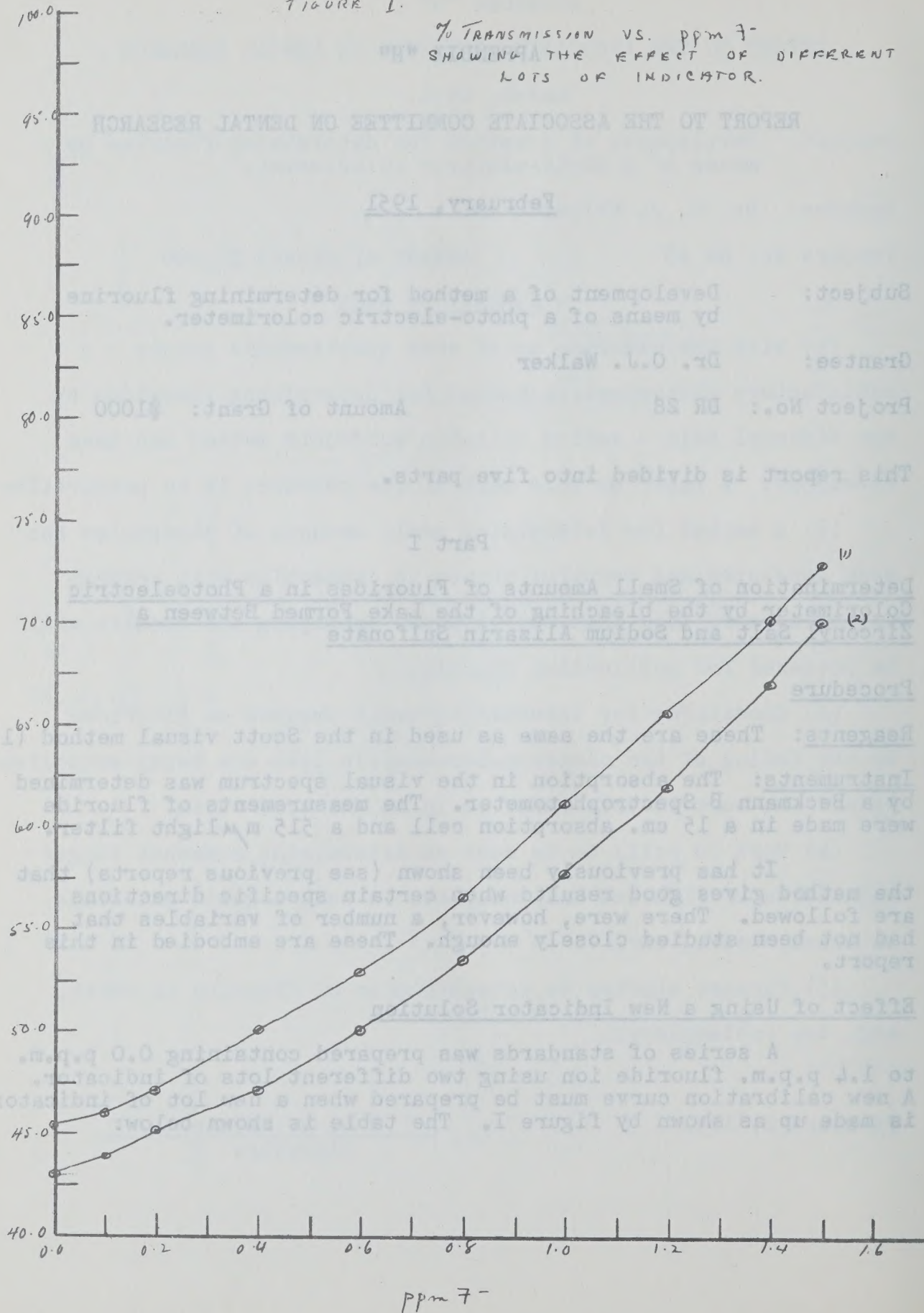




Table I

| p.p.m. F <sup>-</sup> | % Transmission |               |
|-----------------------|----------------|---------------|
|                       | Indicator (1)  | Indicator (2) |
| 0.0                   | 45.4           | 43.0          |
| 0.1                   | 46.0           | 43.8          |
| 0.2                   | 47.1           | 45.1          |
| 0.4                   | 50.0           | 47.2          |
| 0.6                   | 52.9           | 50.0          |
| 0.8                   | 56.5           | 53.5          |
| 1.0                   | 61.1           | 57.7          |
| 1.2                   | 65.5           | 61.8          |
| 1.4                   | 70.0           | 66.8          |
| 1.5                   | 72.7           | 69.8          |

Range 45.4 to 72.7 = 27.3 43.0 to 69.8 = 26.8

## 2. The Effect of Time on Results Obtained

If the standards are left in the colorimeter for a short period of time over the regular time of one hour, a difference in per cent transmission occurs. A series of curves (figure II) was prepared using various definite time intervals (stop watch) of: reading immediately after making up; three minutes after; one hour; and two hours after. The two hour set was untimed between each solution, but with the one hour set, an interval of three minutes was allowed between both making up the standards and in reading them. Table II shows the effect of time. If a rapid procedure is desired, allow the standards to stand one hour and use a three minute interval, but if time is no criterion, the standards can stand for 24 hours and not be timed.

Table II

| p.p.m. F <sup>-</sup> | % Transmission |           |        |         |
|-----------------------|----------------|-----------|--------|---------|
|                       | Immediate      | 3 minutes | 1 hour | 2 hours |
| 0.0                   | 48.0           | 51.6      | 45.4   | 44.5    |
| 0.1                   | 49.2           | 53.1      | 46.3   | 45.7    |
| 0.2                   | 50.4           | 54.4      | 47.2   | 45.8    |
| 0.4                   | 53.3           | 57.7      | 49.9   | 47.5    |
| 0.6                   | 56.8           | 61.5      | 53.0   | 50.3    |
| 0.8                   | 60.1           | 66.0      | 56.7   | 52.6    |
| 1.0                   | 66.0           | 70.2      | 61.3   | 57.0    |
| 1.2                   | 70.2           | 73.9      | 65.6   | 60.1    |
| 1.4                   | 73.5           | 78.4      | 70.2   | 64.8    |

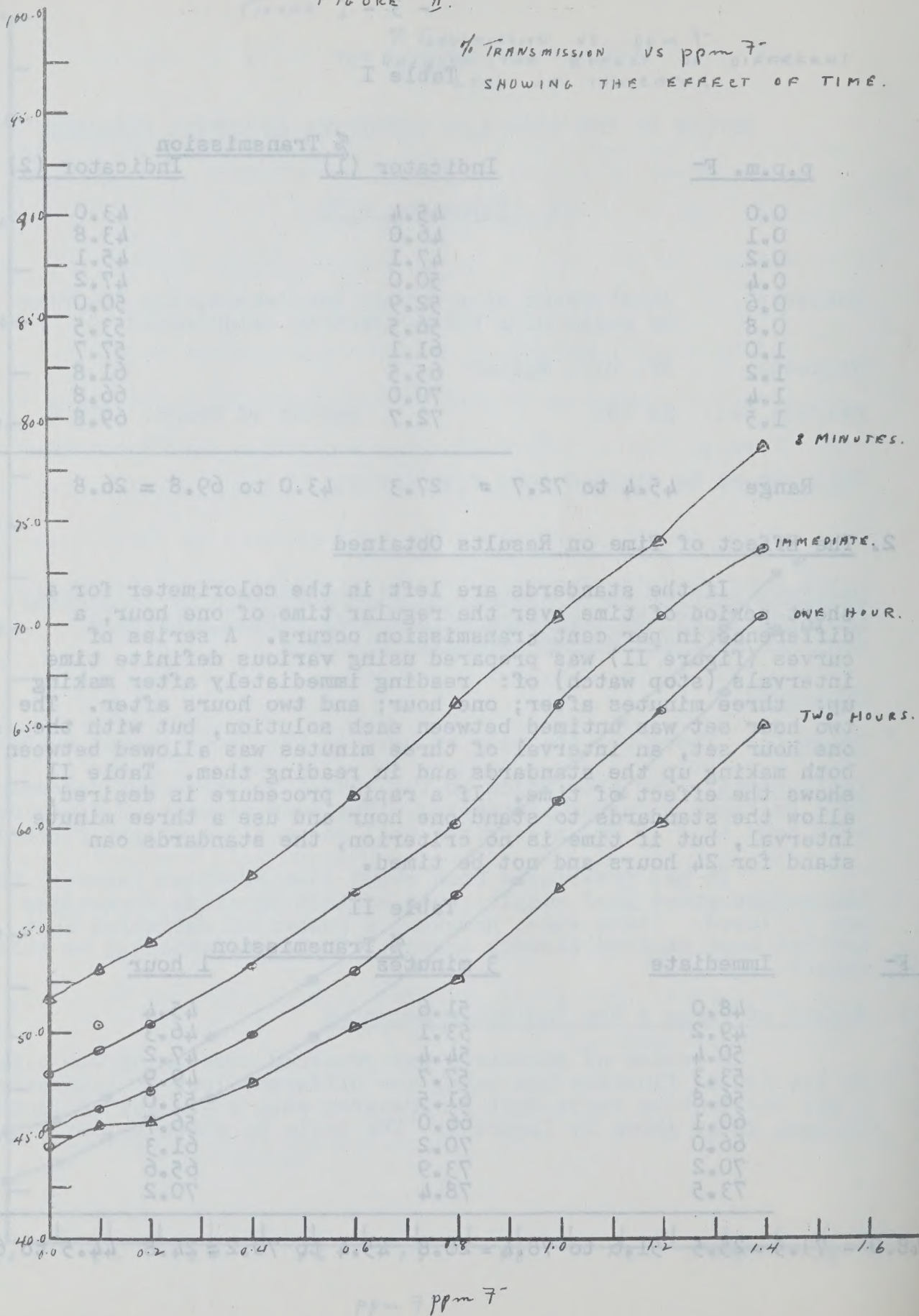
ge 48.0 - 73.5 = 25.5 51.6 to 78.4 = 26.8 45.4 to 70.2 = 24.8 44.5 to 64.8 = 20.3

FIGURE II.

% TRANSMISSION vs ppm  $F^-$

SHOWING THE EFFECT OF TIME.

% TRANSMISSION.





The rate of change, if read three minutes after being made up, is too rapid to get good duplications of results, but the one hour set can be read and duplicated.

The calibration curve showing per cent transmission vs. parts per million fluoride ion is shown in figure III.

### 3. The Effect of Sulfates

These have been considered in an earlier report.

### 4. The Effect of Phosphates

Phosphates interfere with the determination of fluorides in all colorimetric methods so far developed. It has been found here, that up to 3 p.p.m. phosphate ion (as  $\text{KH}_2\text{PO}_4$ ) can be added to standard fluoride solutions without any error larger than the experimental error occurring.

If more than 3 p.p.m. phosphate ion are added, an error up to 0.3 p.p.m. is caused in an extreme case. It will be noted that the fluorine measured is less than had been added, not more, as expected. The least interference is caused when the concentration of fluoride ion is small in relation to the phosphate ion which will normally be the case in water analysis.

See Table III

Table III

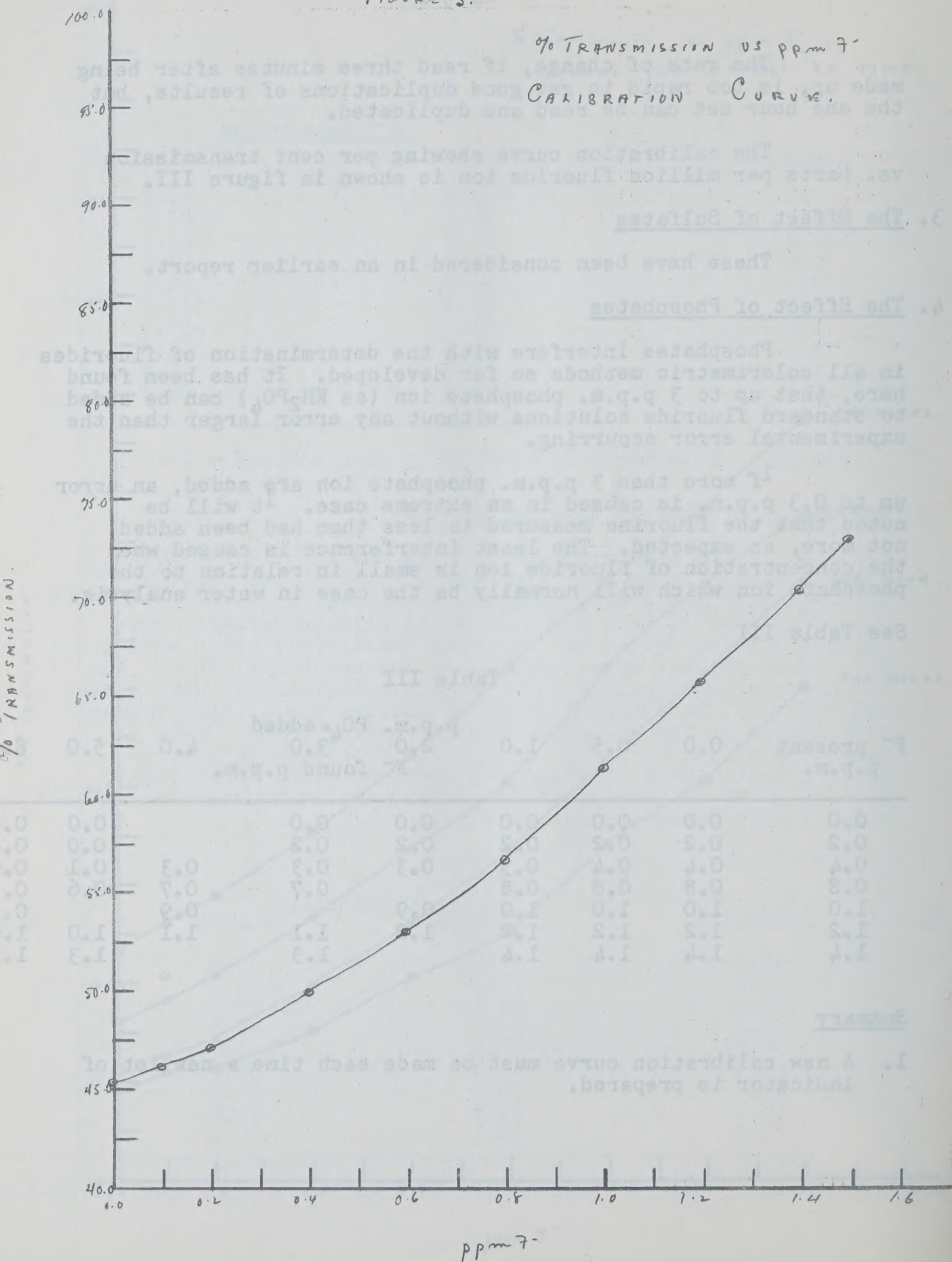
| F <sup>-</sup> present<br>p.p.m. | p.p.m. PO <sub>4</sub> =added<br>F <sup>-</sup> found p.p.m. |     |     |     |     |     |     |     |
|----------------------------------|--------------------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|
|                                  | 0.0                                                          | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 8.0 |
| 0.0                              | 0.0                                                          | 0.0 | 0.0 | 0.0 | 0.0 |     | 0.0 | 0.0 |
| 0.2                              | 0.2                                                          | 0.2 | 0.2 | 0.2 | 0.2 |     | 0.0 | 0.0 |
| 0.4                              | 0.4                                                          | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.1 | 0.0 |
| 0.8                              | 0.8                                                          | 0.8 | 0.8 |     | 0.7 | 0.7 | 0.6 | 0.5 |
| 1.0                              | 1.0                                                          | 1.0 | 1.0 | 0.9 |     | 0.9 |     | 0.8 |
| 1.2                              | 1.2                                                          | 1.2 | 1.2 | 1.2 | 1.1 | 1.1 | 1.0 | 1.0 |
| 1.4                              | 1.4                                                          | 1.4 | 1.4 |     | 1.3 |     | 1.3 | 1.1 |

### Summary

1. A new calibration curve must be made each time a new lot of indicator is prepared.

FIGURE 3.

% TRANSMISSION vs ppm F<sup>-</sup>  
CALIBRATION CURVE.





2. Time is a very important factor in this determination, but good duplications are possible if the standards are allowed to stand one hour before reading and if a three minute interval is used between making up each standard and in reading each standard.
3. Phosphates do not interfere when present in concentrations less than and including 3 p.p.m. The effect of phosphates above 3 p.p.m. is erratic.

## Part II

### Determination of Small Amounts of Phosphates in a Photoelectric Colorimeter Based on Stoloff's Method (2)

When one is dealing with the fluoride content of waters, bones, teeth, etc., a rapid method for determining phosphates is necessary. In an earlier report (October 1950) it was stated that a fairly satisfactory method had been developed, but there were a number of matters that required clarification.

#### Procedure

Reagents: These are the same as used in Stoloff's method except the ammonium molybdate solution.

Ammonium Molybdate Solution: 25 gms. ammonium molybdate, 100 ml. 5N NaOH made up to one litre with 1N H<sub>2</sub>SO<sub>4</sub>. Prepared fresh each week.

Instruments: As with the fluoride determination, a Lumetron Photoelectric Colorimeter, using a 15 cm. absorption cell, was used. A 440 m $\mu$  light filter was used.

#### 1. The pH of the Standards

The pH of the standard solutions is very important for reproducible results. The correct pH was found to be 4.3 and this was obtained by dissolving the ammonium molybdate in 100 ml. 5N NaOH and then making up to one litre with 1N H<sub>2</sub>SO<sub>4</sub>. Fifty grams of ammonium molybdate is 2.4 times in excess of the amount needed so that twenty-five grams is sufficient and prevents precipitation of the molybdate in solution upon standing.

Samples of known phosphate ranging from 0.0 p.p.m. to 6.0 p.p.m. were made up and the average results of five runs are given in Table IV and the calibration curve in figure IV.



Table IV

| p.p.m. $\text{PO}_4^{3-}$ | Average % Transmission | $\log_{10}$ % Transmission |
|---------------------------|------------------------|----------------------------|
| 0.0                       | 100.0                  | 2.0                        |
| 0.2                       | 93.9                   | 1.97                       |
| 0.5                       | 84.8                   | 1.93                       |
| 1.0                       | 72.1                   | 1.86                       |
| 1.5                       | 59.9                   | 1.78                       |
| 2.0                       | 50.8                   | 1.71                       |
| 2.5                       | 42.3                   | 1.63                       |
| 3.0                       | 35.7                   | 1.55                       |
| 4.0                       | 25.7                   | 1.41                       |
| 5.0                       | 18.2                   | 1.26                       |
| 6.0                       | 14.0                   | 1.15                       |

The curve obtained plotting per cent transmission vs. p.p.m. phosphate ion is hyperbolic in nature, and by plotting  $\log_{10}$  per cent transmission vs. p.p.m. phosphate ion (figure V) it is shown that the curve closely follows Beer's Law.

## 2. Effect of Fluorides

Table V

| $\text{PO}_4^{3-}$ present<br>p.p.m. | 0.0 | 0.2 | 0.5 | 1.0                                                   | 1.5 | 2.0 | 3.0 | 5.0  | 7.0 | 8.0 | 10.0 |
|--------------------------------------|-----|-----|-----|-------------------------------------------------------|-----|-----|-----|------|-----|-----|------|
|                                      |     |     |     | p.p.m. $\text{F}^-$ added<br>$\text{PO}_4^{3-}$ found |     |     |     |      |     |     |      |
| 0.0                                  | 0.0 | 0.0 | 0.0 | 0.0                                                   | 0.0 | 0.0 | 0.0 |      |     |     |      |
| 0.5                                  | 0.5 |     |     | 0.6                                                   |     |     | 0.7 | 0.8  |     |     | 1.0  |
| 1.0                                  | 1.0 | 1.0 | 1.0 | 1.0                                                   | 1.0 | 1.1 | 1.1 | 1.1  |     |     | 1.3  |
| 2.5                                  | 2.5 |     | 2.5 | 2.5                                                   | 2.5 | 2.5 | 2.6 | 2.65 |     |     | 2.8  |
| 4.0                                  | 4.0 |     |     |                                                       |     |     | 4.0 |      | 4.2 | 4.2 | 4.3  |
| 5.0                                  | 5.0 |     |     |                                                       |     |     | 5.0 |      | 5.2 | 5.2 | 5.5  |

## 3. Efficiency of Borate Ion in Removing Fluoride Ion

It had been reported previously that boric acid forms fluoroborate ion with the fluoride ion, and therefore, prevents the interference caused by the fluoride ion in the phosphate determination. It has been found here however, that boric acid is not effective in typing up the fluoride ion as shown in Table VI.



- 9 -  
FIGURE IV

% TRANSMISSION vs. ppm  $PO_4^{3-}$   
CALIBRATION CURVE.

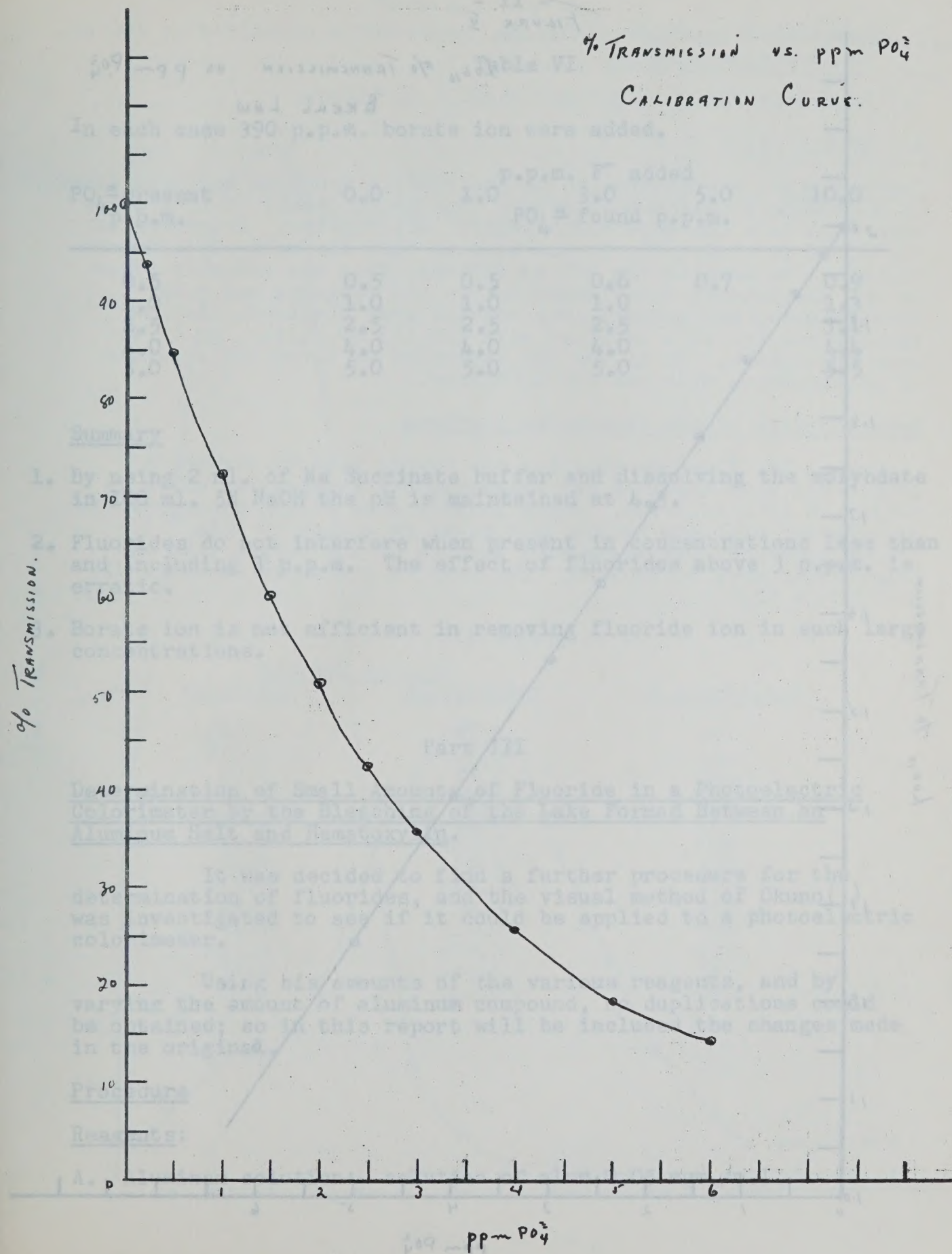
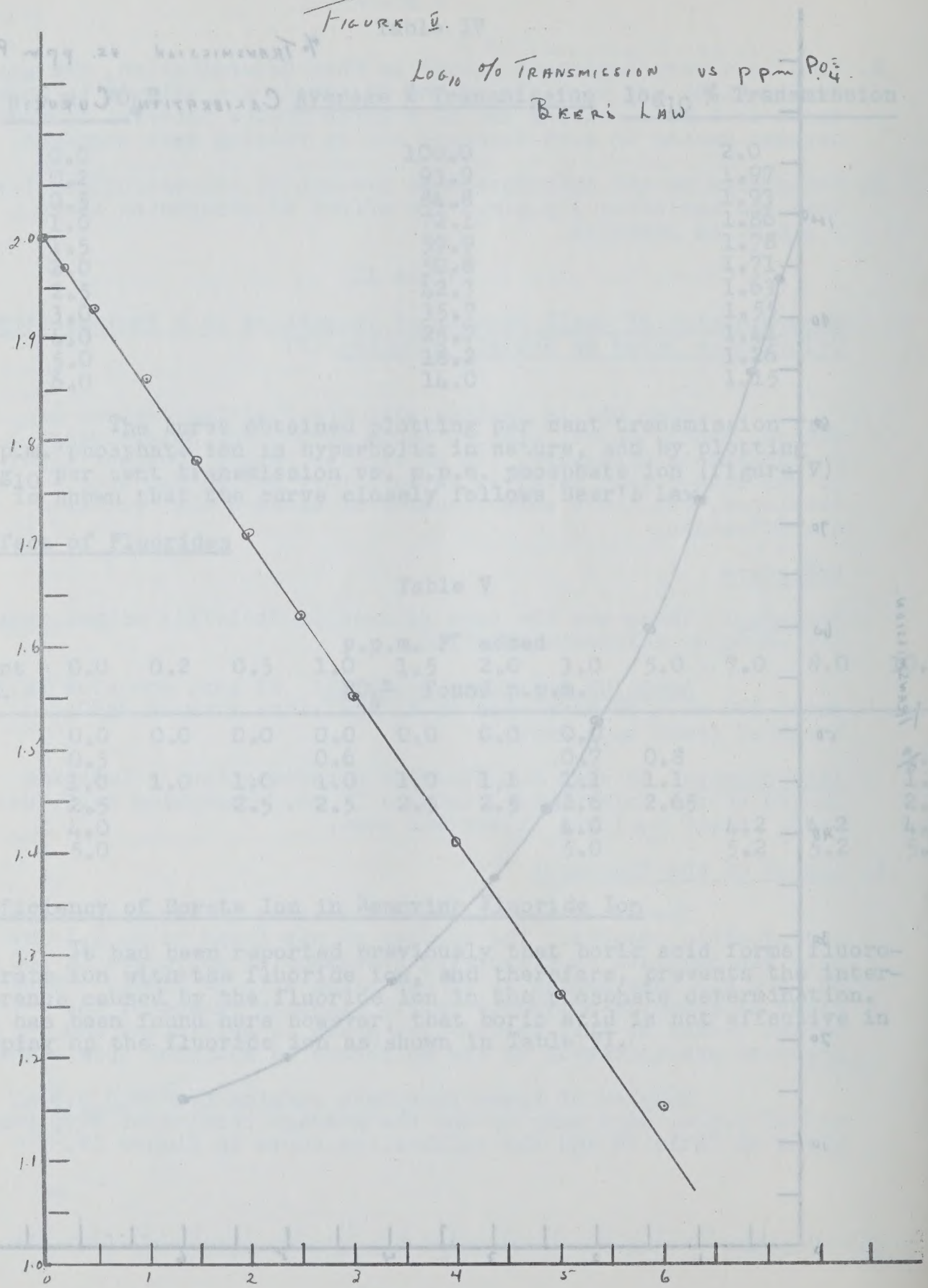


FIGURE II.

LOG<sub>10</sub> % TRANSMISSION VS ppm PO<sub>4</sub><sup>3-</sup>

BEER'S LAW

LOG<sub>10</sub> % TRANSMISSION



ppm PO<sub>4</sub><sup>3-</sup>



Table VI

In each case 390 p.p.m. borate ion were added.

| PO <sub>4</sub> <sup>=</sup> present<br>p.p.m. | p.p.m. F <sup>-</sup> added               |     |     |     |      |
|------------------------------------------------|-------------------------------------------|-----|-----|-----|------|
|                                                | 0.0                                       | 1.0 | 3.0 | 5.0 | 10.0 |
|                                                | PO <sub>4</sub> <sup>=</sup> found p.p.m. |     |     |     |      |
| 0.5                                            | 0.5                                       | 0.5 | 0.6 | 0.7 | 0.9  |
| 1.0                                            | 1.0                                       | 1.0 | 1.0 |     | 1.3  |
| 2.5                                            | 2.5                                       | 2.5 | 2.5 |     | 3.1  |
| 4.0                                            | 4.0                                       | 4.0 | 4.0 |     | 4.4  |
| 5.0                                            | 5.0                                       | 5.0 | 5.0 |     | 5.5  |

### Summary

1. By using 2 ml. of Na Succinate buffer and dissolving the molybdate in 100 ml. 5N NaOH the pH is maintained at 4.3.
2. Fluorides do not interfere when present in concentrations less than and including 3 p.p.m. The effect of fluorides above 3 p.p.m. is erratic.
3. Borate ion is not efficient in removing fluoride ion in such large concentrations.

### Part III

#### Determination of Small Amounts of Fluoride in a Photoelectric Colorimeter by the Bleaching of the Lake Formed Between an Aluminum Salt and Hematoxylin.

It was decided to find a further procedure for the determination of fluorides, and the visual method of Okuno(3) was investigated to see if it could be applied to a photoelectric colorimeter.

Using his amounts of the various reagents, and by varying the amount of aluminum compound, no duplications could be obtained; so in this report will be included the changes made in the original.

### Procedure

#### Reagents:

A. Aluminum solution: solution of alum 0.05 mgm./ml.

- B. Hematoxylin solution: 0.1 gm. hematoxylin dissolved in 100 ml. of 1% acetic acid.
- C. Saturated  $\text{NaHCO}_3$  solution:  $\text{NaHCO}_3$  in excess.
- D. Acetic acid: 1:2

Instruments: The absorption in the visible spectrum was determined by a Beckman B Spectrophotometer. The measurements of fluoride were made in a Lumetron Photoelectric Colorimeter. According to the curve in figure VI, it can be seen that the most satisfactory filter to be used is the 550 m $\mu$  filter. This was actually tried in the preliminary experiments before the reagents had been decided upon, and inconsistent results were obtained. Satisfactory results were obtained with the 515 m $\mu$  filter, so it was used throughout. It is now our intention to carry out a series of measurements with the 550 m $\mu$  filter.

# 1. Determination of the Absorption Spectrum

The fluorine bleaches the purple lake by forming a stable ion with aluminum and releasing the hematoxylin which is yellow in acid solution. Since interference in the photoelectric colorimeter with the purple lake might be caused by this yellow compound, the absorption spectra of the purple lake and hematoxylin were determined. The results are shown in Table VII and figure VI.

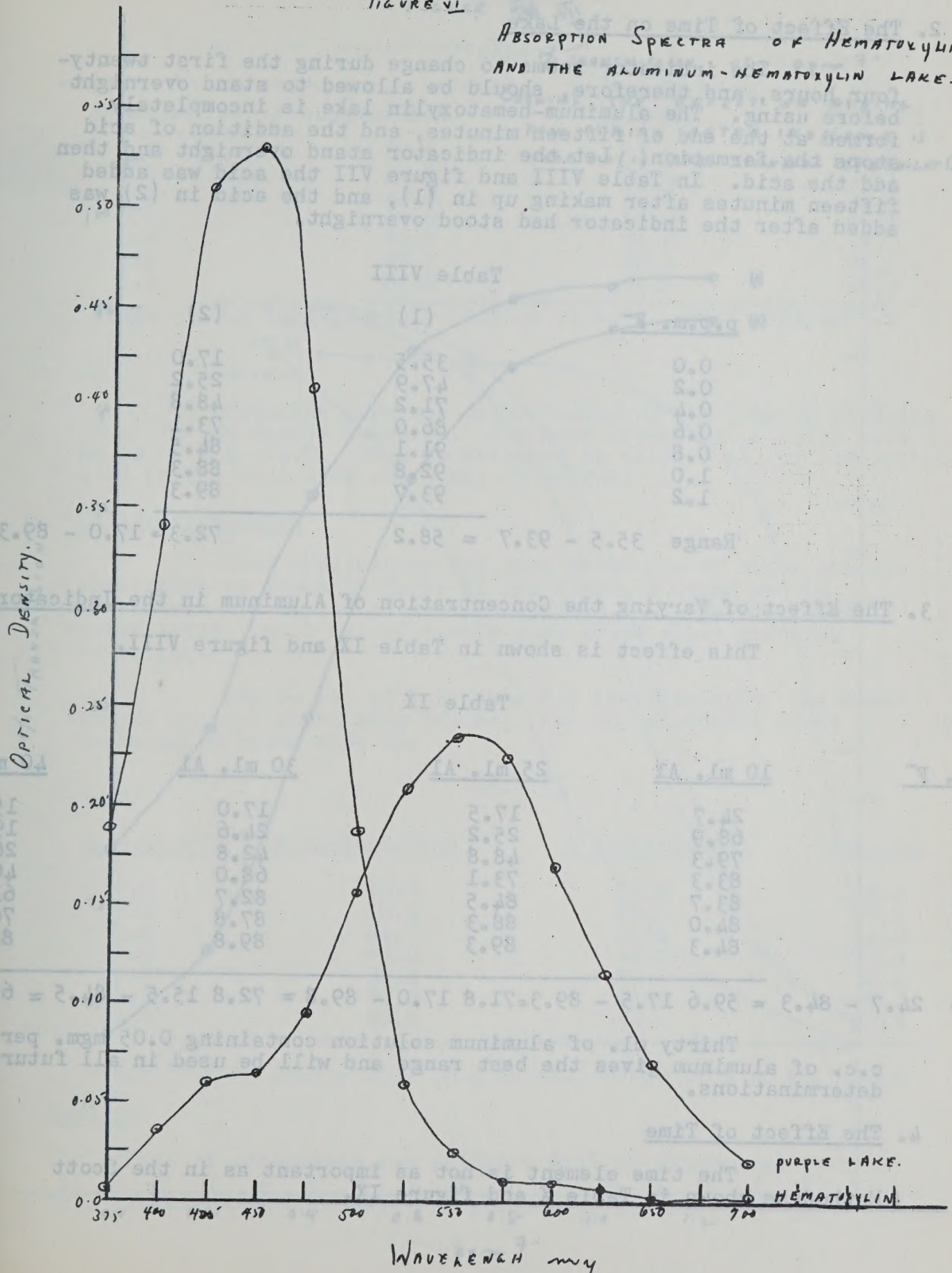
Table VII

| <u>Wavelength m<math>\mu</math></u> | <u>Optical Density</u> |                  |
|-------------------------------------|------------------------|------------------|
|                                     | <u>Hematoxylin</u>     | <u>Indicator</u> |
| 375                                 | 0.188                  | 0.006            |
| 400                                 | 0.34                   | 0.036            |
| 425                                 | 0.51                   | 0.06             |
| 450                                 | 0.53                   | 0.065            |
| 475                                 | 0.41                   | 0.095            |
| 500                                 | 0.185                  | 0.157            |
| 525                                 | 0.06                   | 0.21             |
| 550                                 | 0.025                  | 0.235            |
| 575                                 | 0.01                   | 0.225            |
| 600                                 | 0.01                   | 0.17             |
| 625                                 | 0.006                  | 0.115            |
| 650                                 | 0.002                  | 0.07             |
| 700                                 | 0.001                  | 0.02             |



FIGURE VI

# ABSORPTION SPECTRA OF HEMATOKYLIN AND THE ALUMINUM-HEMATOKYLIN LAKE.



## 2. The Effect of Time on the Lake

The indicator seems to change during the first twenty-four hours, and therefore, should be allowed to stand overnight before using. The aluminum-hematoxylin lake is incompletely formed at the end of fifteen minutes, and the addition of acid stops the formation. Let the indicator stand overnight and then add the acid. In Table VIII and figure VII the acid was added fifteen minutes after making up in (1), and the acid in (2) was added after the indicator had stood overnight.

Table VIII

| p.p.m. $F^-$ | (1)                | (2)                |
|--------------|--------------------|--------------------|
| 0.0          | 35.5               | 17.0               |
| 0.2          | 47.9               | 25.2               |
| 0.4          | 71.2               | 48.8               |
| 0.6          | 86.0               | 73.1               |
| 0.8          | 91.1               | 84.5               |
| 1.0          | 92.8               | 88.3               |
| 1.2          | 93.7               | 89.3               |
| Range        | 35.5 - 93.7 = 58.2 | 72.3 = 17.0 - 89.3 |

## 3. The Effect of Varying the Concentration of Aluminum in the Indicator

This effect is shown in Table IX and figure VIII.

Table IX

| p.p.m. $F^-$ | 10 ml. Al | 25 ml. Al | 30 ml. Al | 40 ml. |
|--------------|-----------|-----------|-----------|--------|
| 0.0          | 24.7      | 17.5      | 17.0      | 15.5   |
| 0.2          | 68.9      | 25.2      | 24.6      | 19.3   |
| 0.4          | 79.3      | 48.8      | 42.8      | 26.1   |
| 0.6          | 83.3      | 73.1      | 68.0      | 40.5   |
| 0.8          | 83.7      | 84.5      | 82.7      | 61.0   |
| 1.0          | 84.0      | 88.3      | 87.8      | 76.4   |
| 1.2          | 84.3      | 89.3      | 89.8      | 84.5   |

Range 24.7 - 84.3 = 59.6 17.5 - 89.3 = 71.8 17.0 - 89.8 = 72.8 15.5 - 84.5 = 69.0

Thirty ml. of aluminum solution containing 0.05 mgm. per c.c. of aluminum gives the best range and will be used in all future determinations.

## 4. The Effect of Time

The time element is not as important as in the Scott Method as shown in Table X and figure IX.



FIGURE VII.

% TRANSMISSION VS ppm  $F^-$

SHOWING THE EFFECT OF ADDING  
ACID 15 MINS AFTER INDICATOR IS  
MADE UP (1) AND STANDING OVERNIGHT (2)

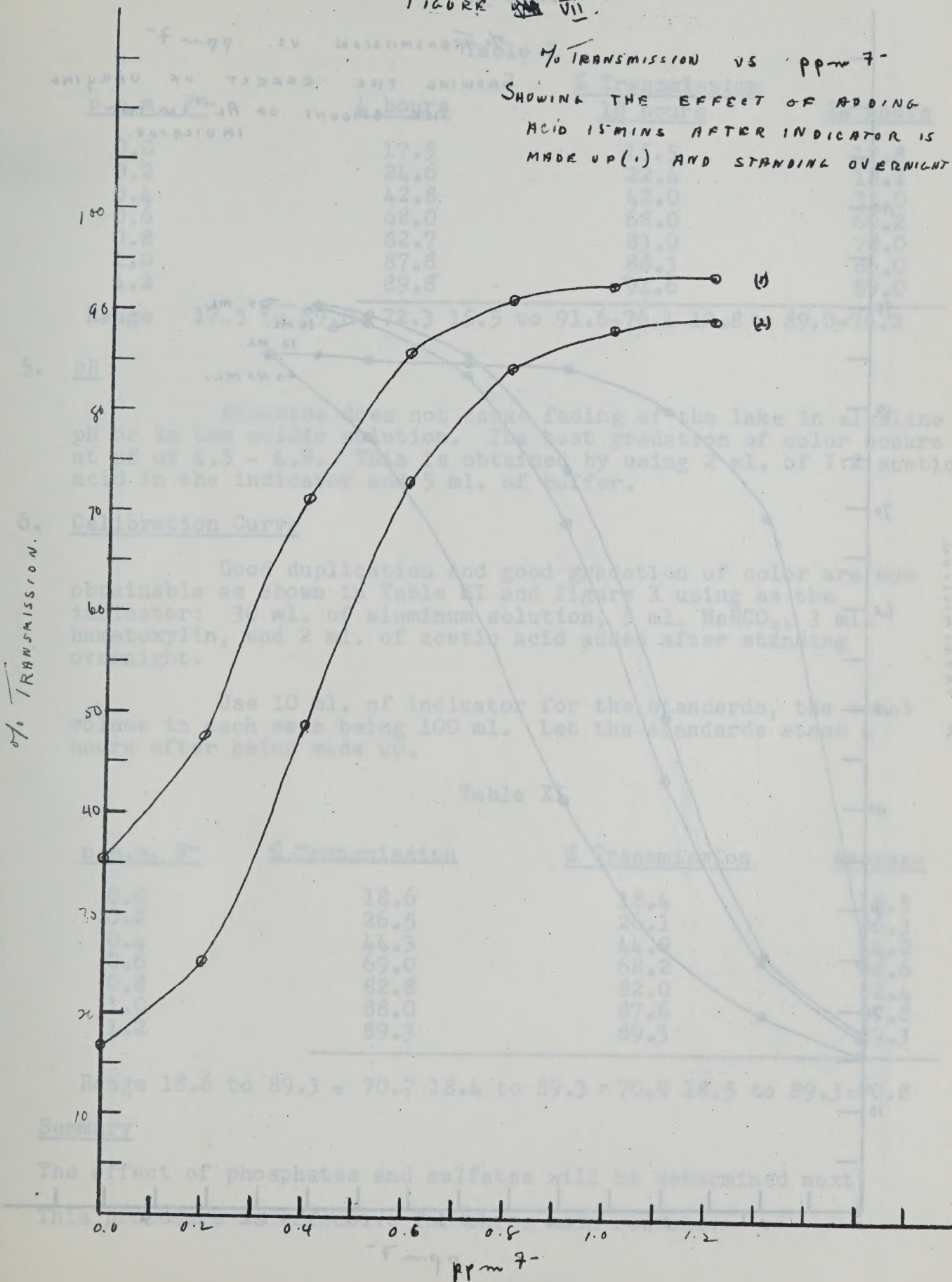


FIGURE VIII.

% TRANSMISSION vs.  $\text{ppm } \text{F}^-$   
 SHOWING THE EFFECT OF VARYING  
 TITR AMOUNT OF  $\text{Al}^{+++}$  IN THE  
 INDICATOR.

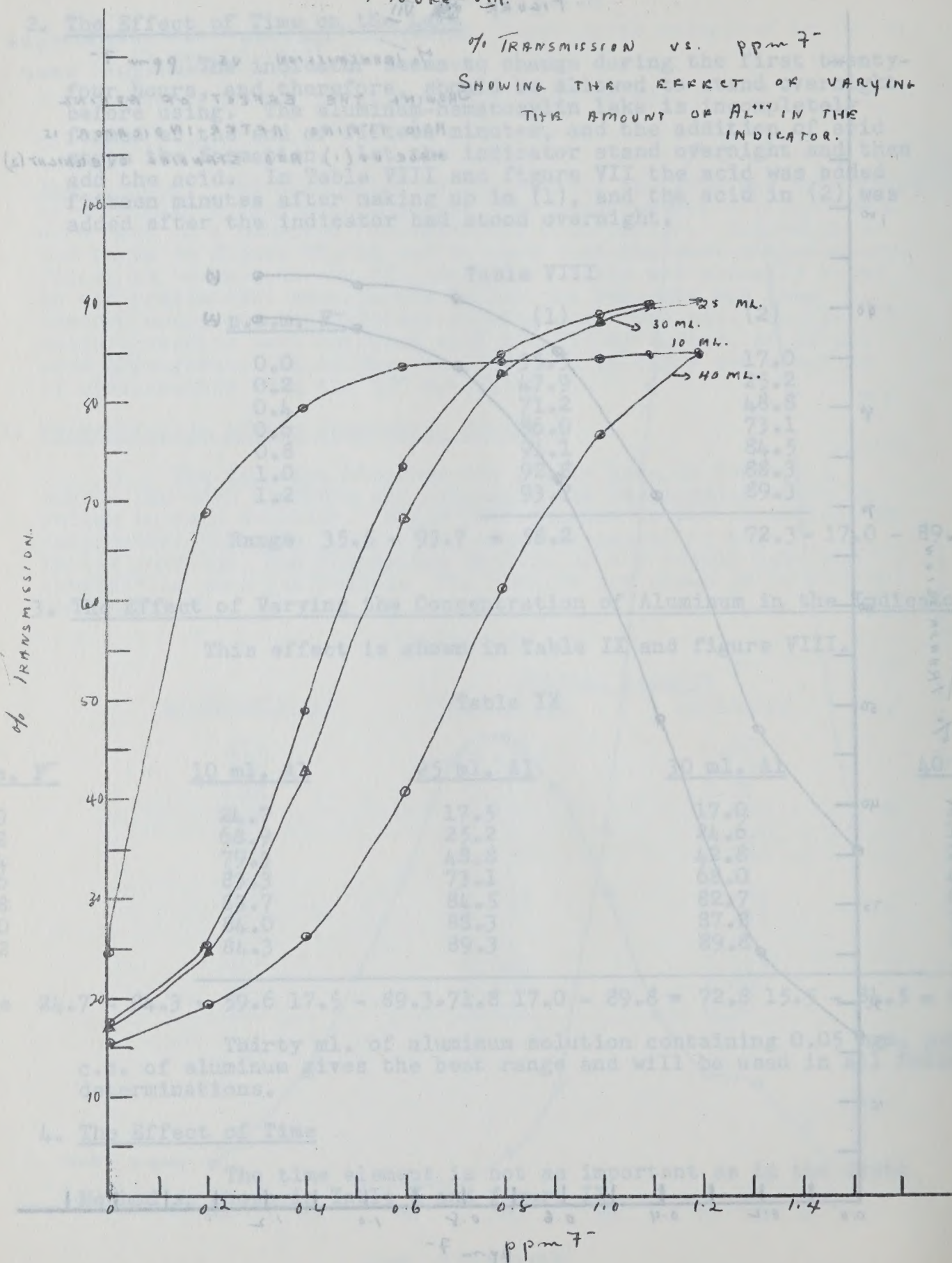




Table X

| <u>p.p.m. F<sup>-</sup></u>                                       | <u>4 hours</u> | <u>% Transmission</u> |                 |
|-------------------------------------------------------------------|----------------|-----------------------|-----------------|
|                                                                   |                | <u>18 hours</u>       | <u>24 hours</u> |
| 0.0                                                               | 17.5           | 15.5                  | 12.8            |
| 0.2                                                               | 24.6           | 22.4                  | 18.1            |
| 0.4                                                               | 42.8           | 42.0                  | 35.0            |
| 0.6                                                               | 68.0           | 68.0                  | 60.2            |
| 0.8                                                               | 82.7           | 83.0                  | 78.0            |
| 1.0                                                               | 87.8           | 88.3                  | 86.0            |
| 1.2                                                               | 89.8           | 91.6                  | 89.0            |
| Range 17.5 to 89.8 = 72.3 15.5 to 91.6 = 76.1 12.8 to 89.0 = 76.2 |                |                       |                 |

5. pH

Fluoride does not cause fading of the lake in alkaline pH or in too acidic solution. The best gradation of color occurs at pH of 4.5 - 4.8. This is obtained by using 2 ml. of 1:2 acetic acid in the indicator and 5 ml. of buffer.

6. Calibration Curve

Good duplication and good gradation of color are now obtainable as shown in Table XI and figure X using as the indicator: 30 ml. of aluminum solution, 5 ml. NaHCO<sub>3</sub>, 3 ml. hematoxylin, and 2 ml. of acetic acid added after standing overnight.

Use 10 ml. of indicator for the standards, the total volume in each case being 100 ml. Let the standards stand 4 hours after being made up.

Table XI

| <u>p.p.m. F<sup>-</sup></u> | <u>% Transmission</u> | <u>% Transmission</u> | <u>Average</u> |
|-----------------------------|-----------------------|-----------------------|----------------|
| 0.0                         | 18.6                  | 18.4                  | 18.5           |
| 0.2                         | 26.5                  | 26.1                  | 26.3           |
| 0.4                         | 44.3                  | 44.0                  | 44.2           |
| 0.6                         | 69.0                  | 68.2                  | 68.6           |
| 0.8                         | 82.8                  | 82.0                  | 82.4           |
| 1.0                         | 88.0                  | 87.6                  | 87.8           |
| 1.2                         | 89.3                  | 89.3                  | 89.3           |

Range 18.6 to 89.3 = 70.7 18.4 to 89.3 = 70.9 18.5 to 89.3 = 70.8

Summary

The effect of phosphates and sulfates will be determined next.

This procedure is effective for three main reasons:

FIGURE 1X

SHOWING THE EFFECT OF  
TIME ON THE STANDARDS.

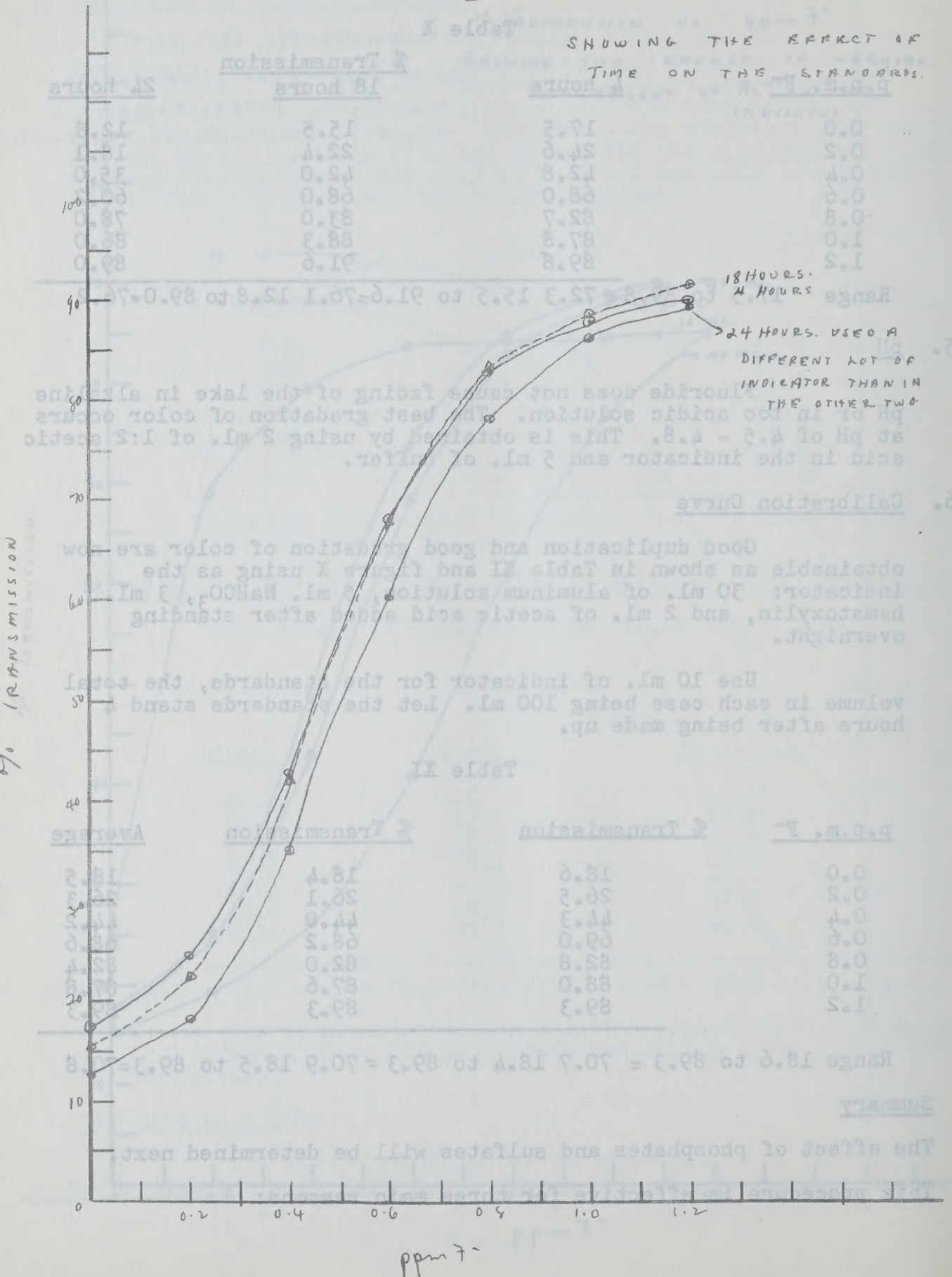
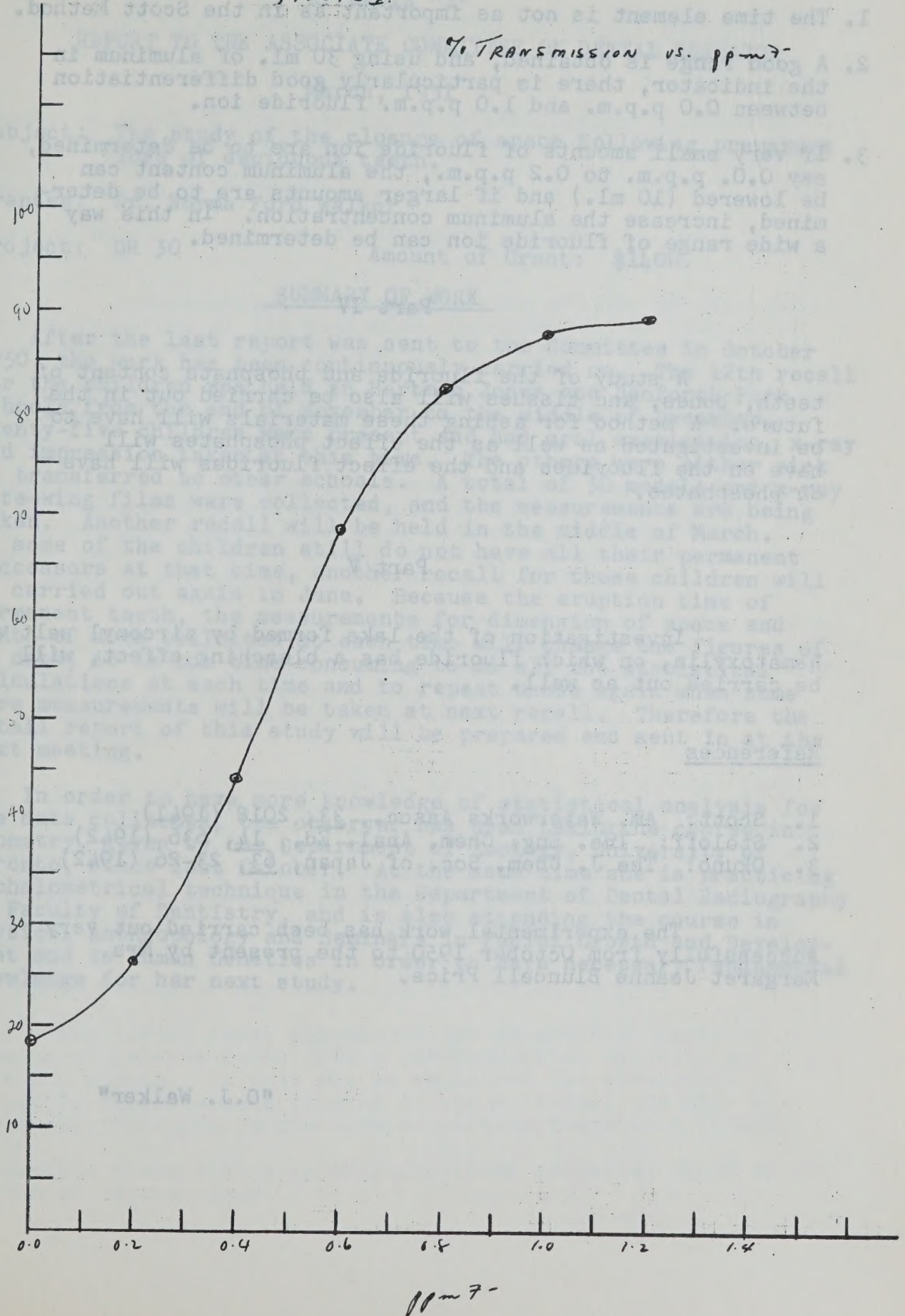




FIGURE 1

% TRANSMISSION vs. ppm F<sup>-</sup>



1. The time element is not as important as in the Scott Method.
2. A good range is obtained, and using 30 ml. of aluminum in the indicator, there is particularly good differentiation between 0.0 p.p.m. and 1.0 p.p.m. fluoride ion.
3. If very small amounts of fluoride ion are to be determined, say 0.0 p.p.m. to 0.2 p.p.m., the aluminum content can be lowered (10 ml.) and if larger amounts are to be determined, increase the aluminum concentration. In this way a wide range of fluoride ion can be determined.

#### Part IV

A study of the fluoride and phosphate content of teeth, bones, and tissues will also be carried out in the future. A method for ashing these materials will have to be investigated as well as the effect phosphates will have on the fluorides and the effect fluorides will have on phosphates.

#### Part V

Investigation of the lake formed by zirconyl salt and hematoxylin, on which fluoride has a bleaching effect, will be carried out as well.

#### References

1. Scott: Am. Waterworks Assoc., 33, 2018 (1941)
2. Stoloff: Ins. Eng. Chem. Anal. Ed., 14, 636 (1942)
3. Okuno: The J. Chem. Soc. of Japan, 63, 23-26 (1942)

The experimental work has been carried out very successfully from October 1950 to the present by Mrs. Margaret Jeanne Blundell Price.

"O.J. Walker"



## APPENDIX "I"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951

Subject: The study of the closure of space following premature loss of deciduous teeth.

Grantee: Dr. Norma Ford Walker

Project: DR 30

Amount of Grant: \$1400.

#### SUMMARY OF WORK

After the last report was sent to the Committee in October 1950, the work has been continuously carried on. The 12th recall for the children was held in Hartman Jones and Danforth Park schools from the end of November to the middle of December. Twenty-five children were present and had oral examination, x-ray and impression taken at this time. The others were either sick or transferred to other schools. A total of 50 models and x-ray bite-wing films were collected, and the measurements are being taken. Another recall will be held in the middle of March. If some of the children still do not have all their permanent successors at that time, another recall for those children will be carried out again in June. Because the eruption time of permanent teeth, the measurements for dimension of space and width of arch collected at each time will change the figures of my data, it is too time-consuming to do all the statistical calculations at each time and to repeat those again when some more measurements will be taken at next recall. Therefore the detail report of this study will be prepared and sent in at the next meeting.

In order to have more knowledge of statistical analysis for the data collected, the observer has been taking the course in Biometry, given by the Department of Zoology, University of Toronto, since last October. At the same time she is practicing cephalometrical technique in the Department of Dental Radiography of Faculty of Dentistry, and is also attending the course in Physical Anthropology and Seminars in Facial Growth and Development and in Human Genetics in order to have necessary fundamental knowledge for her next study.





APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: A study of the calcifying potentialities of calcium oleate on the tissues of the dental pulp.

Grantee: Dr. H. A. Hunter

Project: DR 33 Amount of Grant: \$1500.

I would like to change the name of this project to:-  
"The Investigation of the Mechanism Concerned in the Deposition of Lime Salts Formed in Bridging Over a Pulp Exposure".

Purpose of Project: To develop a means of treating exposed pulps by inducing bridging of the gap in the dentine with a calcified material.

Introduction: True heterotopic bone formation has been observed only in tissues experiencing pathologic changes and containing young connective tissue, i.e. eyes, meninges, tonsils, lungs, thyroid, ovaries, uterine tubes, kidneys, artery walls, wound scars, muscles and tendons - Weinmann and Sicher.

We know also that in healthy and diseased vital pulps, calcium salts are precipitated showing widely different histological appearances. These calcified masses range from normal histological appearing dentine to concentrically laminated calcified masses forming denticles, to cementum and cancellous bone - Kronfeld, Orban, and Euler.

As to the mechanism of deposition of calcium, Adami says:- "The chemical process underlying this appears in some cases at least, to be that a degeneration of cells is accompanied by the liberation of fatty acids which combine with the calcium in the lymph to form compound calcium soaps. In this combination the weaker fatty acids are phosphoric and carbonic acids with the subsequent deposit of insoluble phosphate and carbonate in dead tissues."

Factors in favour of the formation of calcium soaps:-

(1) Virchow (1858) first propounded the metastatic theory of deposits of calcium salts from a supersaturated solution in the blood stream. In this way he explained the occurrence in old people of calcareous plaques in the arteries. He also observed calcification in the form of calcium soaps in a lipoma.

(2) Jaekle found that a calcifying lipoma contained 29.5% of its calcium as calcium soap.



(3) Klotz obtained staining reactions in calcifying tissues suggesting soaps which he extracted by solvents. He urged that as the first step in pathological calcification that the calcium was first laid down as soaps, afterwards undergoing a transformation into the less soluble phosphate and carbonate.

(4) Fischler and Gross obtained microchemical reactions for soaps in margins of infarcts and atheromatous plaques, but not in caseous areas. They considered that calcium soap formation was an important, but not essential step in pathological calcification.

(5) It is a well known fact that calcification occurs chiefly where fatty degeneration has occurred, i.e. in tubercles, atheromatous plaques and also, that in cases of fat necrosis, fatty acids are formed which combine with calcium to form calcium soaps.

On the other hand, Wells, studying large quantities of material chemically, found at most doubtful traces of calcium soaps in calcifying matter, even in the earliest stages, and also very small amounts of other soaps or fatty acids, and therefore questions the occurrence of calcium soaps as an essential step in calcification, although not doubting that under certain conditions, e.g. calcifying lipomata and fat necrosis, this may occur. He goes on to say that it seems probable that there are no essential differences between the processes of ossification and pathological calcification and there seems to be as yet no reason for assuming that in the former calcium soaps constitute an essential step in the process.

Others feel that there are general and local causes for pathological calcification, no one of which is operative in every example. The general causes are hypercalcaemia and hyperphosphataemia, and the local causes are increased alkalinity of the tissues, local increase of phosphatase and inorganic phosphates, and preceding fatty degeneration or necrosis - Barr.

In many older deposits of calcium there is gradual conversion to bone. It is assumed that the surrounding fibroblasts undergo metaplasia to osteoblasts, and the subsequent formation of trabecular bone. Between the trabeculae and spicules there is fibrous tissue or typical bone marrow - Moore.

A local increase in the alkalinity of the tissues is believed to occur and it is attributed to the activity of the appropriate cells, be they osteoblasts, odontoblasts or cementoblasts - Weinmann and Sicher. This rise in pH would reduce the solubility of calcium salts, thus it should facilitate its deposition.

The presence of an excess of calcium and phosphate ions helps their precipitation and this excess may be a general hypercalcaemia and hyperphosphataemia, but it can also be accomplished by the local action of phosphatase on organic phosphates. The calcification might then depend on the local alkalinity in the presence of phosphatase.



Procedure: In order to test these theories and their application to the dental pulp, the following plan has been drawn up.

The theory of unsaturated fatty acids having an affinity for calcium to form calcium soaps and then having the fatty acid radical replaced by inorganic phosphate and carbonate ions, is being tested by placing calcium oleate directly in contact with the exposed pulp tissue and observing the reaction at different periods of time.

Another group of dogs are receiving the same treatment, but substituting cholesterol as the test material.

The question of the influence of pH is being studied by using calcium hydroxide, which has a pH of 12. This material is known to induce calcification, having first been used by Hermann in 1920 and by others since. It might be argued that the calcific properties were due to the calcium ions, so a parallel group of teeth were treated with magnesium hydroxide so as to remove the calcium ions, but retain the hydroxyl ions.

The other three groups of teeth are controls. The Jodo formagen group is included to test this proprietary material whose chief constituent is  $\text{Ca}(\text{OH})_2$ .

Denco temporary cement is the sealing material being used to cover all the test materials and must be included to observe any effects that it might have per se.

The zinc oxide and eugenol mixture is claimed by Zander to have no calcifying propensities and is included to check this claim.

Summary of Work to-date: The experimental work is being done on dogs. The animals are anaesthetized with nembutal and exposures are made of the dental pulps without any attempt to establish a sterile field of operation, nor are the test materials sterilized beforehand. The exposures range from 1 - 2mm. in diameter and were capped as soon as the haemorrhage was controlled. The following tabulated form represents the materials used, the intervals used and the number of teeth in each group. It is planned to extend the number of intervals where it is indicated by the earlier examples.

| <u>Material.</u>       | <u>15 days.</u> | <u>30 days.</u> | <u>60 days.</u> |
|------------------------|-----------------|-----------------|-----------------|
| Calcium oleate         | 4               | 3               | 3               |
| Jodo formagen          |                 | 3               | 2               |
| Zinc oxide- eugenol    |                 | 3               | 2               |
| Calcium hydroxide      |                 | 3               | 4               |
| Denco temporary cement | 4               |                 |                 |
| Magnesium hydroxide    | 3               | 3               |                 |
| Cholesterol            | 3               | 3               |                 |



To-date, three of the above teeth have been serially sectioned and examined; the others are as yet in varying stages of preparation. Of these three teeth, two were treated with Denco temporary cement alone as controls, the third was treated with calcium oleate and oleic acid and, all three were left under treatment for fifteen days.

The pulps of the two controls showed similar histological appearances. Both were vital and showed fairly advanced areolation of the odontoblastic layer, interpreted as an early sign of degeneration. The pulps as a whole showed a well-established reticular degeneration which is felt not to be attributable to the treatment. In one of these teeth, an upper left second molar, there was a moderate regional pulpitis at the site of exposure. There were observed two spicules of dentine separated from the main body and driven into the pulp tissue proper, which showed a peripheral, concentric, hyaline layer of eosin staining material about them. This in turn was bordered by an almost continuous single layer of cells similar to osteogenic cells. There was, however, no sign of any calcified mass being deposited in the area of the exposure.

The tooth treated with calcium oleate in oleic acid showed an overall hyperaemia of the pulp. It differed from the previous two teeth in that there was a narrow, nearly continuous mass of hyaline, eosin staining material stretching across the exposure area and lying superficial to the pulp tissue and beneath a mass of inflammatory cells, which mass was in contact with the calcium oleate-oleic acid mixture.

At this stage of the investigation, the few observations recorded above are considered to be an encouraging lead and will serve to guide the subsequent course of the program.



APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Studies of calcium metabolism in tooth with the aid of  $\text{Ca}^{45}$

Grantee: Dr. A. D'Iorio

Project No. DR 34

Amount of Grant: \$2700.

SUMMARY OF WORK

Introduction -  
Six groups (5 animals) of newly weaned rats are injected intraperitoneally with different doses of  $\text{Ca}^{45} \text{Cl}_2$ . Each group receives daily one injection of 0.4 mg Ca in 1/2 ml solution. These animals receive respectively 1-3-5-7-9-11 injections and are killed 24 hours after the last injection.

The teeth are excised, dried, crushed, sieved and separated into fractions of different densities corresponding to bone cementum dentine and enamel. The specific activity of the Ca precipitated was also increasing in this same order.

By plotting the logarithm of the specific activity against the logarithm of the number of doses it becomes evident that Ca uptake by the tooth in vivo is an adsorption phenomenon described by the Freundlich isotherm. In vitro experiments have also been undertaken to demonstrate whether certain substance such as sugar urea increase the permeability of the tooth towards radiocalcium. Caries free teeth are split and totally embedded in paraffin except for a window of 5x5 mm. These preparations are then incubated at 37°C in a solution containing  $\text{Ca}^{45} \text{Cl}_2$  and the compound to be tested.

No results as yet, are to be reported with this method, but experiments are actually in progress.

"Antoine D'Iorio"

Signature

Date: February 9th, 1951.





## APPENDIX "K"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Studies of calcium metabolism in tooth with the aid of  $\text{Ca}^{45}$

Grantee: Dr. A. D'Iorio

Project No. DR 34

Amount of Grant: \$2700.

#### IN VIVO STUDIES

##### Introduction -

In recent years it has become increasingly clearer that the simplest explanation for the appearance of minerals in the bone is inadequate. This phenomenon is not a simple uptake of Ca and P by the bone, from the blood and extracellular fluids. As stated by Manly, Hodge and Manly, (17) the blood minerals are in some sort of dynamic equilibrium with the corresponding bone minerals.

From in vitro studies (16) (9) (10) with  $\text{P}^{32}$  and  $\text{Ca}^{45}$  it has been shown that the exchange process was an adsorption phenomenon described by the Freundlich isotherm. It was thus logical to verify the exact process of  $\text{Ca}^{45}$  uptake by the tooth in vivo. The following experiment was consequently undertaken to establish the metabolic rate of uptake of Ca in the teeth of young rats.

##### Methods -

Newly weaned male rats weighing approximately 30 gms. are placed in cages with screen wire bottom to avoid coprophagy. Six groups of five animals each\*, receive daily injections of radiocalcium from one to eleven days as indicated in Table I.

---

\* Group 6 includes only four animals





"K-2"

TABLE I

| Group | No injections | Time elapsed between injections | Time elapsed between sacrifice | Radioactivity (c.p.m.) injected to each animal |
|-------|---------------|---------------------------------|--------------------------------|------------------------------------------------|
| 1     | 1             | -                               | -                              | 23750                                          |
| 2     | 3             | 24 hrs.                         | 48 hrs.                        | 71250                                          |
| 3     | 5             | "                               | "                              | 118750                                         |
| 4     | 7             | "                               | "                              | 166250                                         |
| 5     | 9             | "                               | "                              | 213750                                         |
| 6     | 11            | "                               | "                              | 261250                                         |

1.25 gm. of Calcium carbonate containing 0.1 mc.  $\text{Ca}^{45}$  is dissolved in diluted HCl up to 25 ml. 4 ml. are diluted to 100 ml. so that the radioactivity per ml. is 47500 c.p.m. and the total concentration of Ca being 0.8 mgm/ml. The animals are injected daily with 0.5 ml. of this solution intraperitoneally. At the time of sacrifice, animals are anaesthetized and blood is withdrawn by heart puncture. Heads are cut away and fixed in formaline neutralized by Mg.  $\text{SO}_4$ . The rest of the body is dried under vacuum at 100°C and then ashed at 1000°C in a muffle furnace.

The heads are split longitudinally so that one half is preserved for histological treatment. From the other half, teeth are extracted, crushed and sieved through a 100 mesh wire screen. The powdered teeth are separated in different portions according to certain density intervals.

SEPARATION OF THE CALCIFIED PORTION OF THE TOOTH BY THE METHOD OF DIFFERENTIAL DENSITY

Liquids of different densities were prepared according to Hodge and Manly's techniques (14) (15). Table II summarizes the composition of the different liquids and the tooth fractions corresponding to their densities.





"K-3"

TABLE II

| Density | Components                            | (vol.%)      | Fraction isolated    | %purity |
|---------|---------------------------------------|--------------|----------------------|---------|
| 2.70    | Bromoform<br>acetone                  | 91<br>9      | molar<br>enamel      | 99      |
| 2.42    | bromoform<br>acetone                  | 77.6<br>22.4 | incisor<br>enamel    | 99      |
| 2.07    | methyl cellosolve<br>tetrabromoethane | 43.9<br>56.1 | dentine              | 99.7    |
| 1.8     | bromoform<br>acetone                  | 48.1<br>51.9 | cementum and<br>bone | -       |

Isolated fractions are placed in 25 ml. graduated flasks with 5 ml. HCl 20% and boiled for 30 min. in a water bath. 7.5 ml. of  $\text{CCl}_3\text{CO}_2\text{H}$  20% are added and the volume is completed with distilled water. Aliquots of 10 or 20 ml. are treated with ammonium oxalate (pH 5.8) and the calcium oxalate obtained is dried, weighed and uniformly spread on aluminum sample trays for radioactivity measurements. Two ml. aliquots are used for a further dilution i.e. up to 10 ml. One ml. of this final solution is used for calcium determinations using Sobel and Sklarsky's method (19). All determinations are duplicated. Values reported are the mean values of both determinations which should agree within a maximum error of 3%.

#### Results -

By using daily injections, the animals have their calcified tissues regularly enriched with radioactive calcium. Excess of calcium is rapidly withdrawn from the circulating blood, to be fixed in the bones and to a lesser degree the dentine and other calcified tissues. As these experiments are made with very young animals, these phenomena are due to the full strength growing processes. The specific activity of calcium oxalate obtained from the different fractions is reported in Table III. The values indicate a maximum uptake in the fractions of densities ranging from 1.8 to 2.0 i.e. calcifying dentine, cementum and bone. The lower values are found in fully calcified enamel which density exceeds 2.7. Intermediate







#### "K-4"

values are noticeable in fractions ranging from 2.0 to 2.4 and from 2.4 to 2.7, corresponding to fully calcified dentine and calcifying enamel. Tissues of densities less than 1.8 which probably include tendons cartilage and primary spongy bone show the greatest activity in the first three groups, but less than the 1.8-2.0 fraction in the last three ones. As a rule, with the number of injections the increase of specific activity is regular.

A correlation between the percentage uptake of radiocalcium and the number of injections was attempted as indicated in Table IV. The vertical analysis attests that in each fraction the amount of radioactivity is practically constant i.e. directly proportional to the quantity of calcium<sup>45</sup> injected. However, the horizontal analysis established that the differences noted between all fractions may be caused by some factors like specific surface, absolute weight and the potential of dilution in the already calcified structures.

The constancy of the vertical data shows that there is a certain exchange with the blood calcium and that a definite portion of radiocalcium is integrated for each of the different calcified tissues. It appears clearly that it is more active in the bone and cementum (1.8-2.0), where a mean value of 1.14% of the total radioactivity injected is traced back. The lower values found for the others show that the uptake is scarce and slow. About the (1.8) fraction the very low mean value (.19) indicates that, after 24 hours, equilibrium, has already been reached since a long time and according to the high dilutions occurring in the intercellular liquid of those soft tissues, a relatively small uptake is noticeable at this time.

#### Radioautographic Technique -

Radioautographs of gross sections were made from both jaws after a single injection of calcium lactate containing 2,260,000 c.p.m. Sections even in contact 15 days with a N T B I Eastman Plate 100 thick coated with gelatin. Bone is highly radioactive as well as cementum and calcifying dentine especially in the incisors. Details from molars are not so obvious. Sharp images can be obtained only from parallel plane sections. We have already designed an apparatus which we hope will permit us to obtain very thin ground sections.

#### Discussion -

Data from Table III were plotted in Graphics I and II. If Ca uptake by teeth had been a simple absorption phenomenon, as it was previously supposed Graphic I would show straight lines. It is obvious then that this process is not one of the first order which is often the case for metabolic reactions. In Graphic II the straight path of all the curves prove that the exchange described by Freundlich isotherm is still going on in the living organism and that the rate of exchange is almost the same for all fractions. The fact that the last curve is steeper may be easily







explained, if one bears in mind that enamel may adsorb calcium from saliva, thus, two sources of calcium are at the disposal of enamel; first, an endogenous way by mean of dental lymph (5)(6), second, an exogenous way by mean of saliva which continually flushes the tooth crown (18)(2)(3). Hence the ordinate X/M is increasing more rapidly when plotted against the time. As already shown by Johansson in vitro (11).

Indeed, if the general formula is

$\log X/M = \log K + \frac{1}{n} \log C$ , (where X/M is the adsorbed radiocalcium per mgm of total calcium, C is the concentration of the liquid phase and K and n are constants), it can be assumed that values of C for enamel fractions ( 2.7) are influenced by both sources (lymph and saliva).

The results obtained strongly suggest that in vivo the uptake of Ca is an adsorption phenomenon related to the crystal surface (8).

Thus variations of adsorption and crystal surfaces may be expressed as follows:

bone>dentine>enamel

These results confirm in vivo the findings made recently in vitro with powdered bone (9)(16).

#### IN VITRO STUDIES

Several works have proven the fact that enamel is permeable to inorganic as well as to organic substances (1)(12)(13)(14).

In order to measure permeability changes in the presence of some factors, we have set up the following scheme which is under investigation already.

#### Methods -

Human teeth free from caries (impacted molars and impacted bicuspids removed on orthodontic prescriptions) are split on the labio-lingual axis and dehydrated in ethylalcohol 95% neutralized with  $\text{NaHCO}_3$  for 24 hours. Boyd's (7) technique was followed for embedding and windowing. One half serves as control, the other is used to follow permeability changes. Each embedded half is placed in 25 ml. tubes with 10 ml. of a solution containing 20 mgm. of calcium, with a radioactivity of 59375 c.p.m./mg Ca. 500 mgm of glucose is added to one of them. Few crystals of thymol are added to prevent molding. The incubation time is one week at 37°C. After this time, the blocks are washed, enamel of the exposed window is removed and dissolved in HCl 20% for calcium determinations and radioactivity measurements.

Substances to be tested are, glucose, ammonia, urea, chlorophyll etc.

A definite report on this part will probably be available next summer.







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"K-7"

TABLE III

Specific Activity\* of Ca oxalate from different fractions

| Groups | Density of fractions |                |                  |                  |                   |
|--------|----------------------|----------------|------------------|------------------|-------------------|
|        | 1.8                  | 1.8-2.0        | 2.0-2.4          | 2.4-2.7          | 2.7               |
| 1      | C.P.M./mg<br>14.2    | C.P.M./mg<br>9 | C.P.M./mg<br>3.3 | C.P.M./mg<br>3.2 | C.P.M./mg<br>0.95 |
| 2      | 27.2                 | 26.8           | 10.2             | 9.8              | 5.3               |
| 3      | 39.6                 | 34.4           | 15.15            | 11.9             | 6.7               |
| 4      | 36                   | 44.2           | 26.4             | 20.7             | 11.6              |
| 5      | 45                   | 67.5           | 32.7             | 31.2             | 21.9              |
| 6      | 67.2                 | 100.8          | 40.5             | -                | 22.5              |

\* Specific activity = Counts per minute per mg of Calcium oxalate





"K-8"

TABLE IV

Percent of injected dose found in the different fractions

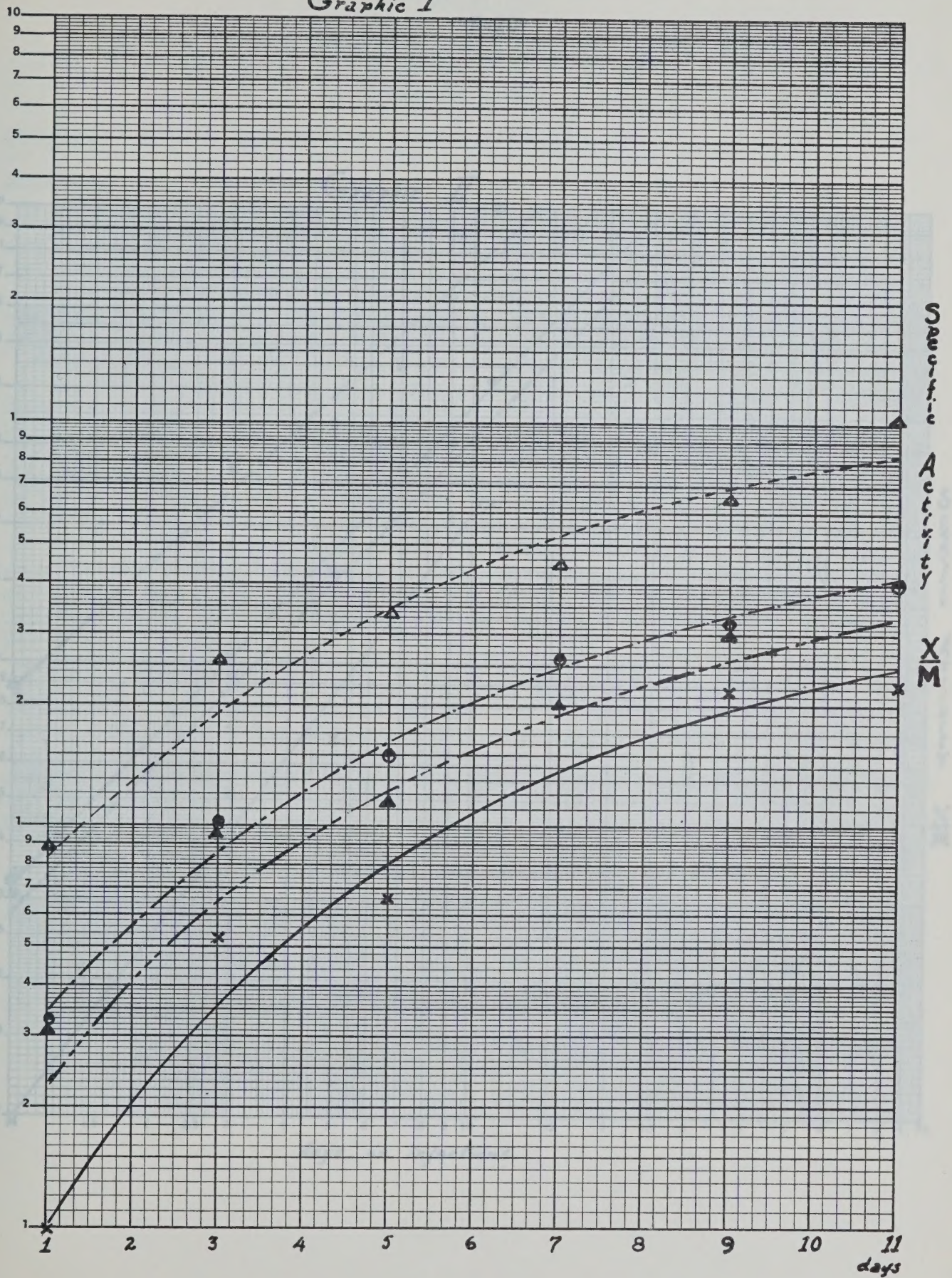
| No injections | Density of fractions |          |          |          |                  |
|---------------|----------------------|----------|----------|----------|------------------|
|               | Less than<br>1.8     | 1.8-2.0  | 2.0-2.4  | 2.4-2.7  | More than<br>2.7 |
|               | %<br>.25             | %<br>.98 | %<br>.23 | %<br>.22 | %<br>.064        |
| 1             |                      |          |          |          |                  |
| 3             | .18                  | 1.33     | .33      | .24      | .17              |
| 5             | .23                  | 1.33     | .24      | .19      | .12              |
| 7             | .12                  | .62      | .37      | .33      | .22              |
| 9             | .10                  | 1.24     | .57      | .16      | .40              |
| 11            | .24                  | 1.34     | .49      | .45      | .35              |
| Mean values   | 0.19                 | 1.14     | 0.37     | 0.26     | 0.22             |

$$\frac{\text{C.P.M./mg Ca oxalate} \times \text{mg Ca oxalate} \times 100}{\text{C.P.M. injected} \times \text{no. of animals}}$$





# Graphic I "K-9"









"K-10"

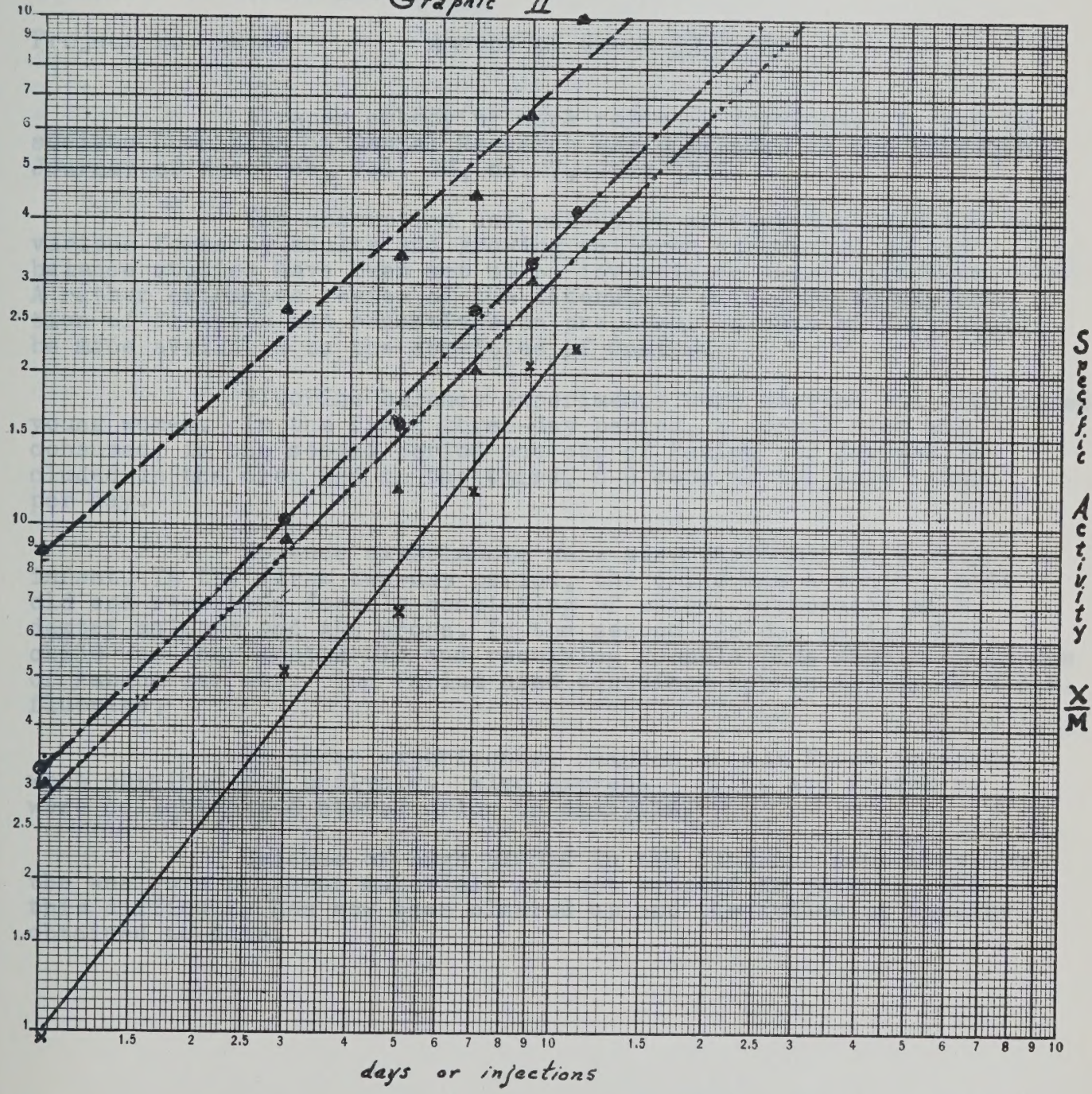
REPORT TO THE ASSOCIATE COMMISSIONER OF THE PUBLIC HEALTH SERVICE

March, 1941.

Subject: Biological absorption of fluorine

Grantee: S. Doreen Sm

Graphic II









## APPENDIX "L"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Biological absorption of fluorine

Grantee: M. Doreen Smith

Project No. DR 29

Amount of Grant \$375.00

A paper embodying work reported previously to the Associate Committee on Dental Research was published in the Canadian Journal of Research, vol. 28, no. 7, July 1950.

The absorption and retention of fluorine ingested in various forms by adults and growing children interested us and balance studies have been set up to investigate these questions. A rather extensive review of the literature referring to absorption of fluorine has recently been prepared. A copy of this could be made available to the committee if desired.

Fluorine balance experiments were carried out with three young women. In the first three-day period, a normal diet was consumed; in the second period one cup of Pabulum per day was included in the diet and in the third, 6-9 cups of tea were drunk per day.

Balance experiments were also performed on a male infant. In the first two-day period, the child was a month a half old and receiving formula consisting of water, evaporated milk, dextri-maltose and ADC drops. In the second two-day period the child was four months old and receiving formula plus nine tablespoons of Pabulum per day. The Pabulum used contained approximately 12 p.p.m. fluorine.

In these experiments, samples of all foods ingested were obtained for analysis. Also excreta - urine and faeces - were collected and stored prior to analysis.

Because of high blanks in the fluorine determination the analysis of these materials did not proceed as planned, but this difficulty has now been cleared up and the following results obtained to-date.



# "L-2"

| <u>Infant</u> | <u>Period I</u> | <u>Material</u>           | <u>Fluorine</u>                 | <u>Average Fluorine</u> |
|---------------|-----------------|---------------------------|---------------------------------|-------------------------|
|               | <u>Intake</u>   | Formula/day               | 73.7, 65.3                      | 69.5                    |
|               | <u>Output</u>   | Faeces (total for 2 days) | 223.6<br>98.3<br>228.9<br>175.3 | 181.5                   |
|               |                 | Faeces/day                |                                 | 90.8                    |
|               |                 | Urine, day I              | 9.7, 19.4                       | 14.6                    |
|               |                 | Urine, day II             | 12.5, 15.9                      | 14.2                    |
|               |                 | Urine/day                 |                                 | 14.4                    |
|               |                 | Total/day                 |                                 | 105.2                   |
|               | Spillage/day    |                           |                                 | 105.2 - 69.5 = 35.7     |

| <u>Infant</u> | <u>Period II</u> | <u>Material</u>           | <u>Fluorine</u>         | <u>Average Fluorine</u> |
|---------------|------------------|---------------------------|-------------------------|-------------------------|
|               | <u>Intake</u>    | Formula/day               | 249.6                   | 249.6                   |
|               |                  | Pablum/day (9 tbsp.)      | 403.2                   | 403.2                   |
|               |                  | Total/day                 |                         | 652.8                   |
|               | <u>Output</u>    | Faeces (total for 2 days) | 140.5<br>348.0<br>594.3 | 360.9                   |
|               |                  | Faeces/day                |                         | 180.4                   |
|               |                  | Urine, day I              |                         | 182.1                   |
|               |                  | Urine, day II             |                         | 134.2                   |
|               |                  | Urine/day                 |                         | 158.1                   |
|               |                  | Total/day                 |                         | 338.5                   |
|               | Storage/day      |                           |                         | 652.8 - 338.5 = 314.3   |

| <u>Adults</u> | <u>Period I</u> | <u>Meals</u>    | <u>Fluorine</u> |
|---------------|-----------------|-----------------|-----------------|
|               |                 | Dinner No. 1    | 21.6            |
|               |                 | Breakfast No. 1 | 131.6           |
|               |                 | Lunch No. 1     | 270.1           |



"L-3"

In the first period, the young child is excreting more fluorine than he is ingesting. This may be due to a large storage of fluorine in the infant because of a high fluorine intake of the mother, perhaps from bonemeal tablets, in the pre-natal period. In the second period, the infant is storing fluorine from that ingested.

As can be seen from these results, we experienced difficulty in obtaining good duplicates with fecal material. Some work has been done on this problem and it appears to be caused by a sampling error. The fecal material is mixed in a Waring blender and considerable foam results. We are now proceeding to break this foam in order to get more homogeneous samples.

The distillation of urine ash is complicated by the necessity of adding a considerable amount of silver sulphate to precipitate the chlorides present. This precipitate causes considerable bumping during distillation and it was hoped that it could be eliminated. Various materials were tested as to their value in dissolving the ash so that the chlorides could then be precipitated and filtered off before distillation, but no suitable dissolving agent was found.

Work is proceeding to solve difficulties concerned with the analysis of fluorine in biological material. Miss Ham is continuing with the investigation of fluorine absorption and retention as a Ph.D. problem and has begun further balance studies on two more infants.

"M. Doreen Smith"

Signature

There is a tendency for the fluorine content of the enamel from Pabulum teeth to be higher than that of the enamel of the non-Pabulum teeth. Also the mean of the dentin to enamel ratio is 1.6 for the Pabulum teeth and 2.6 for the non-Pabulum teeth.

Further analysis of the teeth from Pabulum and non-Pabulum children will be carried out.

The results of the analysis of some Barnis teeth are also included.

Date: February 15, 1951

"M. Doreen Smith"





APPENDIX "M"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Fluoride content of enamel and dentine of teeth  
from Toronto children studied in relation to  
Pablum history of the children's diet

Grantee: M. Doreen Smith

Project No. DR 13

Amount of Grant: \$1475.

Summary of Report

Most of the work on this project prior to November 1st has been reported in a paper entitled "An Examination of the Fluoride Content of Teeth from some Ontario Districts" published in the January 1951 issue of the Canadian Dental Association Journal.

Some difficulty was experienced with the method during the summer, but this has now been cleared up.

The cereal, Pablum, has been found to have an appreciable amount of fluorine. Therefore an examination is being carried out to determine the effect of Pablum ingestion on the fluorine content of the permanent teeth. A collection is being made of permanent teeth of children, five to seventeen years old, who have been ingesting Toronto water (0.1%F) all their lives, and the differences between the Pablum and non-Pablum group are being noted. The enamel and dentin of eighteen of these were analyzed for fluorine content.

There is a tendency for the fluorine content of the enamel from Pablum teeth to be higher than that of the enamel of the non-Pablum teeth. Also the mean of the dentin to enamel ratio is 1.6 for the Pablum teeth and 2.6 for the non-Pablum teeth.

Further analysis of the teeth from Pablum and non-Pablum children will be carried out.

The results of the analysis of some Sarnia teeth are also included.

Date: February 15, 1951

"M. Doreen Smith"





## APPENDIX "M"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Fluoride content of enamel and dentin of teeth  
from Toronto children studied in relation to  
Pablum history of the children's diet

Grantee: M. Doreen Smith

Project No. DR 13                      Amount of Grant: \$1475.00

Most of the work on this project done prior to November 1st has been reported in a paper entitled "An Examination of the Fluoride Content of Teeth from Some Ontario Districts" published in the January 1951 issue of The Journal of the Canadian Dental Association.

During the summer months a great deal of difficulty was experienced with the method. Apparently one of the solutions became contaminated. It was very difficult to track down the source since many solutions were used. An effort was made to trace it to the reagents used in a new research problem started in the department. This could not be proved but the work on the new problem is being carried out in another room and watched carefully. The potassium hydroxide was responsible for the high blanks. The method is once again proceeding satisfactorily.

Miss Harness left for England the end of November and the analytical work is being continued by Mr. W.D. Powrie who worked for a month with Miss Harness before she left.

Although the new process Pablum now on the market contains around 6 p.p.m. fluorine, the old process Pablum was found in this laboratory and by D. J. Martin (1) to contain around 12 p.p.m. The percentage bonemeal in the Pablum has not been changed but the cereal is given a heat treatment which would very probably change the fluorine content. This old Pablum was not recalled from the market until some time in 1949. Therefore if children of the age group of 5 - 17 years were fed Pablum as a cereal during infancy its fluorine content could be assumed to be around 12 p.p.m. An attempt was made to collect extracted teeth from children in this age group noting whether or not they had been fed Pablum, and also limiting the group to those brought up on Toronto water.

(1) Journal of Dental Research, No. 27, No. 1, p. 27, 1948.



Permanent teeth were very difficult to procure in this age range. Deciduous teeth were easily collected but it was felt analyses of the permanent teeth would be of more significance. Many pregnant mothers are fed bonemeal capsules which might influence the fluorine content of deciduous teeth.

Eighty letters were sent to Toronto dentists appealing for teeth extracted for orthodontic reasons. Only two were obtained by this method and it was necessary for Mr. Powrie to follow through with personal visits to obtain teeth. Eighteen more were collected and it is hoped further effort will provide us with enough teeth to make the study of statistical value.

The procedure for preparation of teeth; separation into dentin and enamel; and analyses of fluorine follows that outlined in the paper of Harness and Smith.

The results of analyses of teeth from Pablum fed children are shown in Table I and for those from non-Pablum fed children in Table II. There is a tendency for the fluoride content of the enamel from the Pablum teeth to be higher than in the enamel of the non-Pablum teeth. The mean of the dentin-enamel ratio is 1.6 for the teeth in Table I and 2.6 for the teeth in Table II. However more analysis may very likely change this ratio, especially as five of the teeth in Table I were from two children who continued to eat Pablum as a cereal right through childhood.

McLure (2) observed in 1941 that the fluorine content of the molar teeth of experimental rats is changed in direct proportion to the amount of fluorine in the diet. However, it could not be determined from these experiments whether the action of fluorine was local, systemic or associated with tooth structure.

Dean (3) in 1942, showed that the fluorine content of mature rat molars is increased by increasing the fluorine content of the drinking water. This increase apparently is in the enamel alone and does not affect the dentin.

The fact that the teeth analysed include molar, bicuspids and incisors introduces further variables since as McClure (4) suggests: different developmental and calcification characteristics of various functional types of teeth might introduce variations in the amount of fluorine acquired by the teeth, particularly as they develop and calcify.

(2) McLure, F.J., Journal of Nutrition, 22:391, 1941

(3) Dean U.S., Public Health, 57, 1942

(4) McLure, J.F., J.D. Res., 27:287, 1948.



"M-3"

Table III shows the results of analyses of some of the teeth from Sarnia which are in the process of being analysed.

Table I - Pablum fed children

| No. | Age | Sex | Type of tooth<br>(Permanent) | Degree of<br>Caries   | Dentin<br>%F | Enamel<br>%F | D/E |
|-----|-----|-----|------------------------------|-----------------------|--------------|--------------|-----|
| 1   | 5   | F   | 1st lower molar              | slight<br>moderate    | 0.007        | 0.005        | 1.4 |
| 2   | 10  | M   | 1st lower molar              | severe<br>large       | 0.011        | 0.006        | 1.8 |
| 3   | 11  | M   | 2nd upper molar              | severe                | 0.008        | 0.005        | 1.6 |
| 4#  | 12  | M   | 1st upper<br>bicuspid        | no decay              | 0.012        | 0.011        | 1.1 |
| 5#  | 12  | M   | 1st upper<br>bicuspid        | no decay              | 0.014        |              |     |
| 6#  | 12  | M   | lower central<br>incisor     | no decay              | 0.005        | 0.004        | 1.2 |
| 7#  | 12  | M   | lower central<br>incisor     | no decay              | 0.006        | 0.003        | 1.7 |
| 8#  | 13  | F   | 1st bicuspid                 | no decay              | 0.011        | 0.006        | 1.8 |
| 9#  | 13  | F   | 1st bicuspid                 | no decay              | 0.008        | 0.004        | 2.0 |
| 10  | 13  | F   | lower rt.molar               | large decay<br>severe | 0.006        | 0.003        | 2.0 |

Mean ratio of D/E = 1.6

# No. 4 - 7 from same mouth; ate Pablum up to 11 years of age

No. 8 - 9 from same mouth; ate Pablum up to 12 years of age

"M-4"

Table II - Non-Pablum fed children

| No. | Age | Sex | Type of Tooth<br>(Permanent) | Degree of<br>Caries | Dentin<br>%F | Enamel<br>%F | D/E |
|-----|-----|-----|------------------------------|---------------------|--------------|--------------|-----|
| 11  | 6   | F   | 1st upper molar              | slight<br>moderate  | 0.009        | 0.003        | 3.0 |
| 12  | 6   | F   | 1st upper molar              | slight<br>moderate  | 0.010        | 0.002        | 5.0 |
| 13  | 6   | F   | 1st molar                    | slight<br>moderate  | 0.008        | 0.003        | 2.6 |
| 14# | 10  | F   | secondary<br>bicuspid        | no decay            | 0.011        | 0.009        | 1.1 |
| 15# | 10  | F   | secondary<br>bicuspid        | no decay            | 0.010        | 0.005        | 1.8 |
| 16  | 15  | F   | lower central<br>incisor     | no decay            | 0.013        | 0.005        | 2.6 |
| 17# | 25  | F   | upper<br>incisor             | no decay            | 0.047        | 0.022        | 2.3 |
| 18# | 25  | F   | upper<br>incisor             | no decay            | 0.049        | 0.018        | 2.8 |

Mean ratio of D/E = 2.6

# No.14 and 15 from same mouth  
No.17 and 18 from same mouth

- (2) McLaren, W.J., Journal of Nutrition, 22:391, 1941  
(3) Dean, W.S., Public Health, 57, 1942  
(4) McLaren, W.J., J.D. Res., 27:287, 1946.



Table III - Sarnia Teeth

| No. | Age | Tooth Analyzed        | Degree of Caries | Enamel % Fluoride | Dentine % Fluoride | Ratio of Dentine to Enamel |
|-----|-----|-----------------------|------------------|-------------------|--------------------|----------------------------|
| 1   | 10  | upper right 1st molar | moderate         | 0.002             | 0.005              | 2.5                        |
| 2   | 12  | upper right 1st molar | moderate         | 0.021             | 0.045              | 2.1                        |
| 3   | 14  | upper left 1st molar  | severe           | 0.008             | 0.0203             | 2.5                        |
| 4   | 14  | lower left bicuspid   | moderate         | 0.004             | 0.012              | 3.0                        |

15 February 1951.

M. Doreen Smith

Table I summarizes the data gathered to date. These readings were obtained by means of a physiologic pressure transducer (Statham Laboratories model 123-A) used in conjunction with a strain gauge analyzer (Brush) and ink writing kymograph (Brush). A hydrostatic system, involving a latex car, adapted to the thumb, finger, etc. under study, and a small caliber hard rubber tube, transmits the applied force to the recording apparatus. Many readings are taken on each subject. Table I makes use of only the average values for each subject.

Several interesting points arise. No habit has yet been studied whose force against the dentition and/or palate was not well above the physiologic limits of capillary pressure (i.e. 25 gms./sq. cm.) The greatest forces applied are those of thumbsucking and the sucking of index and middle finger simultaneously. The position of the hand has a bearing on the amount of force exerted. When the palm is up, the weight is usually much greater.





## APPENDIX "N"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Subject: A study of the normal and abnormal physiologic forces in the oral cavity

Grantee: Robert E. Moyers

Project: DR 26

Amount of Grant: \$150.

As was previously noted, work on this project has been broken down into three separate studies. The progress of each will be reported separately.

#### A. Measurement of some of the sucking habits said to be detrimental to the growth of the dentition

The dental, medical, psychologic, and lay literature is full of many unverified statements about the influence of certain sucking habits on the development of the dentition. It has been previously established (Schwartz<sup>3</sup>, Moyers<sup>1</sup>) that pressures against the teeth greatly in excess of the normal capillary blood pressure will cause osseous changes resulting in tooth movement. This study was undertaken to measure the force of these habits against the teeth and palate. The facts then at hand should tell us whether or not the applied forces are in a range which might cause damage. Further the data might indicate why some of these habits appear detrimental and others seem to do no damage.

Table 1 summarizes the data gathered to date. These readings were obtained by means of a physiologic pressure transducer (Statham Laboratories model 123-A) used in conjunction with a strain gauge analyzer (Brush) and ink writing oscillograph (Brush). A hydrostatic system, involving a latex sac, adapted to the thumb, finger, etc. under study, and a small caliber hard rubber tube, transmits the applied force to the recording apparatus. Many readings are taken on each subject. Table 1 makes use of only the average values for each subject.

Several interesting points arise. No habit has yet been studied whose force against the dentition and/or palate was not well above the physiologic limits of capillary pressure (i.e. 25 gms./sq. cm.) The greatest forces applied are those of thumb sucking and the sucking of index and middle finger simultaneously. The position of the hand has a bearing on the amount of force exerted. When the palm is up, the weight is usually much greater.







These findings are not yet to be taken as a complete analysis. However, we hope to be able to report in the literature on this phase of the work within the next several months. Dr. Donald Hult is assisting in this part of the study.

- B. The measurement of the extent of use of the palatal and pharyngeal muscles in operated and non-operated cleft palate individuals

The method which is proving so accurate in other applications is cumbersome in this instance. We have almost abandoned the effort and are studying the problem by means of electromyographic techniques. The latter method is more satisfactory, but less quantitative. Dr. Ernest Hixon is carrying on the bulk of this work.

- C. The measurement of the forces inherent in the standard orthodontic appliances.

As reported earlier, there is a wide range of forces employed in the various orthodontic mechanisms. Some operate, at times, within the physiologic limits of the tissues. Others are so great that it has been necessary to order an additional strain gauge in order that one instrument could cover the entire range of force application. The first strain gauge used had a range of 0-8 oz. The new instrument will cover 0-24 oz. with slightly less accuracy than at present; the error being, in any gauge,  $\pm .1$  of 1% of the total range of the gauge.

Figure 1 is a bar graph depicting the data thus far gathered. Note that the top limits of the gauge are reached in three of the appliances tested.

There is a variance in the amount of force against different teeth, in the case of mechanisms involving labial arch wires. Table 2 summarizes our data with regard to the edgewise steel mechanism. Note that the greatest forces are applied to the teeth least able to withstand extreme pressures, viz. the central and lateral incisors. Whenever torque, second order, or third order bends are placed in the archwire the forces are in the upper limits of the range. Table 3 supplies similar data for the Johnson Twin Wire Appliance. The forces here are much less than in the Angle edgewise appliance, yet they are well above the desired force range.

Several factors other than sheer weight seem to operate in tooth movements, e.g. the distance the force is active and the time the force is applied. Thus a light force moving over a great distance may do as much damage as a heavy force over a lesser distance. Similarly, though one may bite with a force of







many pounds, it is for a short time only. Many of these other factors have been reported (Moyers<sup>1</sup>, Moyers and Bauer<sup>2</sup>). This study concerns itself with measuring accurately the forces themselves, since such data are lacking in the literature.

The data reported herein are not to be regarded as complete; however, it shows the trend of the findings to date. This part of the study should be ready for reporting in the literature within the next several months. Dr. E. E. Johns is assisting in this part of the study.

TABLE I - SUCKING HABITS

|                            | (N)  | Minimum<br>(grams) | Maximum<br>(grams) | Mean<br>(grams) |
|----------------------------|------|--------------------|--------------------|-----------------|
| Pollex                     |      |                    |                    |                 |
| upwards                    | (14) | 62.2               | 190.1              | 140.2           |
| down                       | (7)  | 41.8               | 149.9              | 126.8           |
| sidewise                   | (3)  | 57.6               | 167.7              | 134.7           |
| Index finger               |      |                    |                    |                 |
| up                         | (2)  | 61.8               | 119.1              | 90.5            |
| down                       | (4)  | 51.8               | 127.1              | 98.6            |
| Index and<br>middle finger |      |                    |                    |                 |
| up                         | (1)  | -                  | -                  | 166.2           |
| down                       | (4)  | 64.6               | 165.5              | 117.2           |
| Tongue thrust              | (27) | 58.1               | 140.0              | 90.1            |
| Lower lip                  | (7)  | 36.1               | 88.9               | 66.8            |

TABLE 2 - EDGEWISE MECHANISM (STEEL)

|              | (N)  | Minimum<br>(grams) | Maximum<br>(grams) | Mean<br>(grams) |
|--------------|------|--------------------|--------------------|-----------------|
| Maxilla      |      |                    |                    |                 |
| Central      | (40) | 82.6               | 200.0?             | 141.8           |
| Lateral      | (40) | 91.7               | 200.0?             | 146.7           |
| Cuspid       | (50) | 80.2               | 185.7              | 139.9           |
| 1st bicuspid | (50) | 84.1               | 200.0?             | 142.2           |
| 2nd bicuspid | (50) | 86.3               | 180.3              | 146.2           |
| 1st molar    | (50) | 89.6               | 194.6              | 150.0           |
| 2nd molar    | (50) | 82.7               | 170.2              | 142.1           |
| Mandible     |      |                    |                    |                 |
| Central      | (32) | 81.9               | 200.0?             | 141.6           |
| Lateral      | (32) | 83.9               | 200.0?             | 144.2           |
| Cuspid       | (34) | 80.4               | 164.0              | 140.1           |
| 1st bicuspid | (34) | 82.6               | 177.7              | 142.7           |
| 2nd bicuspid | (34) | 94.0               | 174.1              | 151.3           |
| 1st molar    | (34) | 87.1               | 190.8              | 149.6           |
| 2nd molar    | (34) | 83.1               | 169.6              | 145.4           |





"N-4"

TABLE 3 - JOHNSON TWIN WIRE

|           | (N)  | Minimum<br>(grams) | Maximum<br>(grams) | Mean<br>(grams) |
|-----------|------|--------------------|--------------------|-----------------|
| Maxilla   |      |                    |                    |                 |
| Central   | (26) | 15.1               | 84.3               | 41.2            |
| Lateral   | (26) | 18.4               | 89.4               | 50.8            |
| Cuspid    | (20) | 24.7               | 46.4               | 39.6            |
| 1st molar | (26) | 26.2               | 51.9               | 42.8            |
| Mandible  |      |                    |                    |                 |
| Central   | (26) | 15.2               | 82.6               | 43.7            |
| Lateral   | (26) | 19.1               | 101.9              | 59.1            |
| Cuspid    | (21) | 22.6               | 54.8               | 40.3            |
| 1st molar | (26) | 28.3               | 56.1               | 44.5            |

References:

1. Moyers, R. E. The Periodontal Membrane in Orthodontics. Jour. Amer. Dental Assn. 40:22-27, 1950.
2. Moyers, R. E. and J. L. Bauer. The Periodontal Response to Various Tooth Movements. Amer. Jour. Ortho. 36:572-580, 1950.
3. Schwartz, A.M. Tissue Changes Incidental to Orthodontic Tooth Movement. Int. Jour. Ortho. 18: 331-352, 1932.

February 13, 1951.



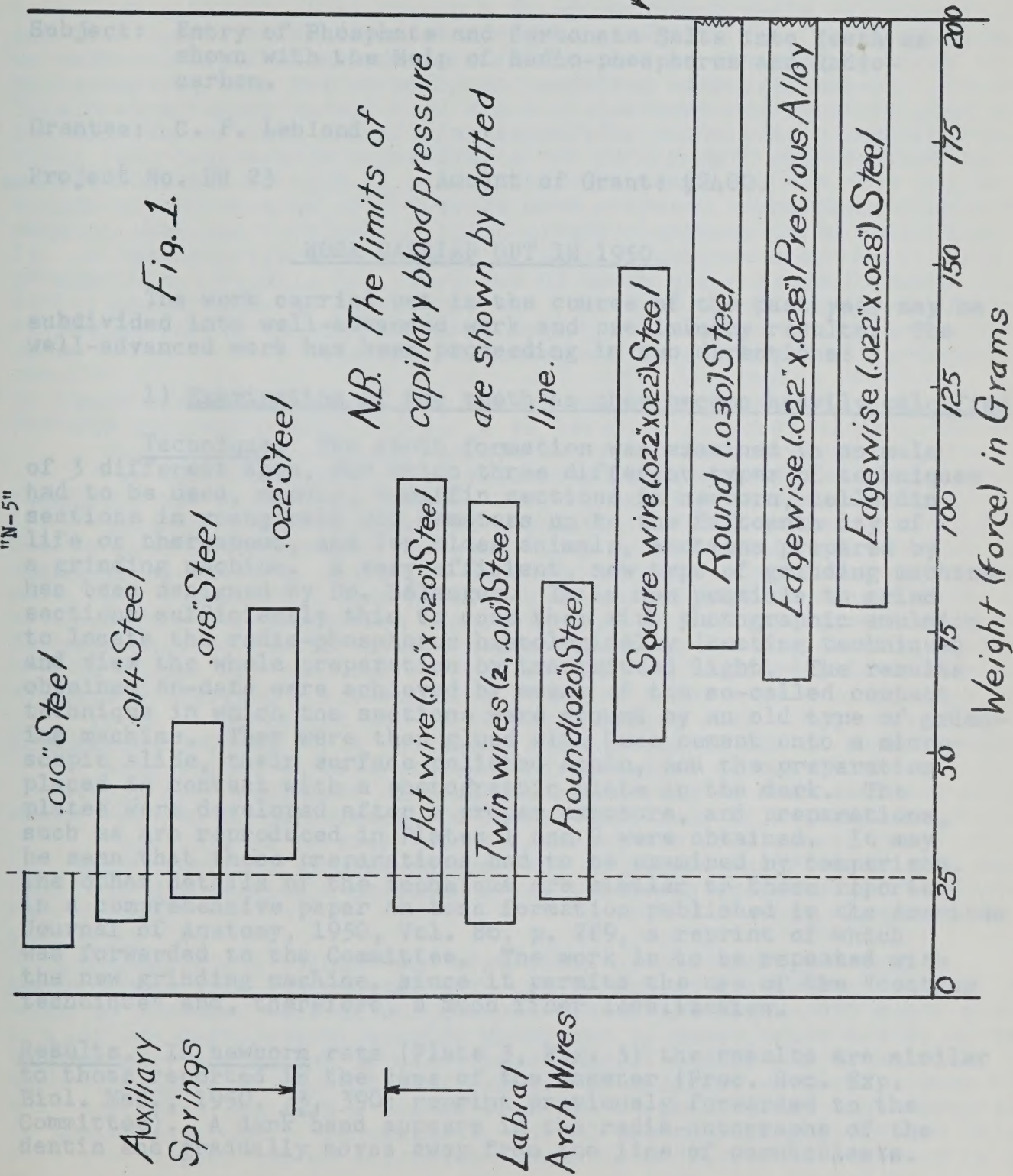


"N-5"

Fig. 1.

Limit of  
present  
Gauge

N.B. The limits of  
capillary blood pressure  
are shown by dotted  
line.







## APPENDIX "O"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: Entry of Phosphate and Carbonate Salts into Teeth as shown with the Help of Radio-phosphorus and Radio-carbon.

Grantee: C. P. Leblond

Project No. DR 23

Amount of Grant: \$2400.

#### WORK CARRIED OUT IN 1950

The work carried out in the course of the past year may be subdivided into well-advanced work and preliminary results. The well-advanced work has been proceeding in two directions:

##### 1) Examination of the teeth as they become heavily calcified

Technique. The tooth formation was examined in animals of 3 different ages, for which three different types of techniques had to be used, namely, paraffin sections in newborn, celloidin sections in young rats and hamsters up to the fifteenth day of life or thereabout, and for older animals, sections prepared by a grinding machine. A very efficient, new type of grinding machine has been designed by Dr. Bélanger. It is now possible to grind sections sufficiently thin to coat them with photographic emulsion to locate the radio-phosphorus histologically (coating technique) and view the whole preparation by transmitted light. The results obtained to-date were achieved by means of the so-called contact technique in which the sections were ground by an old type of grinding machine. They were then glued with Duco cement onto a microscopic slide, their surface polished again, and the preparation placed in contact with a photographic plate in the dark. The plates were developed after a proper exposure, and preparations, such as are reproduced in Plates 1 and 2 were obtained. It may be seen that these preparations had to be examined by comparison. The other details of the technique are similar to those reported in a comprehensive paper on bone formation published in the American Journal of Anatomy, 1950, Vol. 86, p. 289, a reprint of which was forwarded to the Committee. The work is to be repeated with the new grinding machine, since it permits the use of the "coating technique" and, therefore, a much finer localization.

Results. In newborn rats (Plate 3, Fig. 3) the results are similar to those reported in the case of the hamster (Proc. Soc. Exp. Biol. Med., 1950, 73, 390; reprint previously forwarded to the Committee). A dark band appears in the radio-autographs of the dentin and gradually moves away from the line of odontoblasts.



This band is farthest from the odontoblasts at the apex (see the right cusp on fig. 3 of plate 3). In the enamel the radioautographic reaction is a broad and somewhat diffused band which from the earliest times after injection is at a considerable distance from the ameloblasts. In addition, a very thin membrane is present in both rats and hamsters between the apex of the ameloblasts and the enamel. Presumably, this membrane has holes to let cell processes through, although its structure has not been investigated in detail. The important points about this supraameloblastic membrane are its birefringence when viewed with the polarization microscope, its staining with the Von Kossa reaction (plate 3, Fig. 2) and the presence of a radioautographic reaction soon after  $P^{32}$  injection. While the significance of this observation is still in doubt, a tentative interpretation of these results as well as those previously reported is that enamel formation may occur in two steps: 1) elaboration of the thin supra-ameloblastic membrane, the components of which should have a very rapid turnover as judged from the results of the radioautographs; 2) formation of the permanent enamel.

In regard to the dentin, it was previously emphasized that the autographic reaction occurred as a precise band indicating the location of newly-formed dentin crystals. This band is at first close to the odontoblasts and gradually moves away from them as more and more dentin is formed. This observation, which was published in collaboration with Dr. Bélanger in the case of the hamster has now been confirmed in the rat. However, if sufficient time -- let us say ten days -- elapses after an injection of radio-phosphorus it is difficult to find traces of the black band. There are two possible interpretations of this finding. Either sufficient interstitial addition of new phosphate salts occurs (after the time when the circulating level of  $P^{32}$  has fallen) to decrease the amount of radio-phosphorus to a point where it will produce a negligible autographic reaction. This explanation, which was suggested to us by other workers in the field, appears unlikely, since such a process would not sufficiently decrease the concentration of radioactive salts per unit area to prevent an autographic reaction. The alternative explanation is that some degree of dissolution of dentin salts takes place, with the present results tending to indicate that this dissolution occurs at the dentino-enamel junction. Some more work is, however, necessary for a complete clarification of this point.

In young adult and in adult rats the results are illustrated in Plate 1 and Plate 2 respectively. The legends are self explanatory. There was a pronounced difference between the intense reaction of the dentin in the molar teeth of the young animals (Plate 1) and the lack of reaction of the dentin in the molar teeth of the adults (Plate 2). The dentin shows no reaction at all in Plate 2, but the original autograph from which figure 6 was made showed a dentinal reaction which was too faint to appear in the photographs. Therefore, there is no complete interruption of dentin formation in the adult.



## 2) Histochemical studies of teeth

In an attempt to explain the occurrence of newly-formed tooth material as a band close to the odontoblasts in the dentin and as a broad, diffuse band away from the ameloblasts in the enamel, we wanted to know if phosphatase was present in these locations. Much work was done on this subject according to Gomori's method. This method had to be modified since it was necessary to decalcify the material without losing the phosphatase contained in the tissues. Greep and Morse have devised several decalcifying mixtures which produced only a very small loss of phosphatase. Their method was modified in such a way that the sections were cut using undecalcified material and the decalcification was carried out on the slide directly. It was hoped that this process would minimize the loss of phosphatase. At any rate, no phosphatase was detected in either dentin or enamel. On the other hand, the finding of a large amount of phosphatase around the odontoblasts and ameloblasts already reported by Johnson and Bevelander, Greep and Morse was confirmed. These results indicate that the presence of a radioautographic reaction in the midst of the enamel was not caused by an accumulation of phosphatase on the spot.

A number of other histochemical reactions were used, such as the Von Kossa reaction (Plate 3, Figure 2), the trichrome staining (Plate 3, Figure 1) and polysaccharide stains (metachromasia, Hotchkiss reaction, etc.). The staining of the areas giving a radioautographic reaction suggests the possibility that polysaccharide material, because of its viscosity or other properties may hold the newly-formed tooth salts in their position. At any rate, we plan during the coming year to be working on a comprehensive article on the structure of the tooth, in which these various observations would be incorporated.

### Preliminary results

In the course of the past year we have also made an observation which will be used as a lead in future work.

We have emphasized in previous publications, written in collaboration with Dr. Bélanger, that two kinds of radioautographic reactions may be seen in bones and teeth, namely the exchange reactions which are diffuse and occur with appreciable intensity only during the first day following  $P^{32}$  injection, and the reactions due to synthesis of new tooth or bone salts. Presumably, only the phosphate atoms at the periphery of the tooth crystals are liable to exchange, and therefore soon after injection will become radioactive, but they will lose their label by reverse-exchange with non-radioactive phosphate ions as the level of circulating  $P^{32}$  drops. It is presumed that the phosphates contained in the core of the tooth crystals are not exchangeable; and thus when new tooth crystals are formed soon after  $P^{32}$  injection, they will be radioactive throughout and will not lose their label by



"0-4"

exchange. One of the consequences of these facts is that the radioautographic reactions examined soon after injection show diffuse reactions due to exchange, which are sufficiently intense to hide the localized reactions due to new formation of tooth material.

Preliminary results indicate that it is possible to eliminate the reactions due to the  $P^{32}$  entering by exchange. This may be done by fixation in formalin containing a high concentration of phosphate buffer. For instance, by fixing bones of rats sacrificed one hour after  $P^{32}$  injection, the left tibia fixed as usual in neutral formalin (pH 7) lost about 20% of its radioactivity to the fluid, while the right tibia fixed in formalin with phosphate buffers (pH 7) lost about 90% of its radioactivity to the fluid. When the same operation is repeated three days after injection, that is, when there is little or no exchangeable  $P^{32}$  present in the bones, the loss was the same in both solutions. It is believed that, by fixing in a formalin solution buffered with phosphates, it will be possible to clear the tissues from all exchangeable  $P^{32}$ .

This finding may therefore be not only of theoretical but of practical significance.

"C. P. Leblond"



PLATE 1

Explanation of Figures

Coated autographs and ground sections of lower jaws of 50 gm. rats sacrificed at 5 minutes, 2 hours, 2 days and 8 days after injection. The molar teeth above and the incisor tooth below are seen surrounded by bony tissue.

1. Ground section of jaw of animal sacrificed 5 minutes after injection.

2. Contact autograph of figure 1.

The more intense reaction is visible on the inner side of the dentin of the incisor tooth, with a fairly intense reaction on the inner side of the dentin of the molars. A slight reaction of the enamel is barely visible on the lower part of the incisor. A deposition of radio-phosphorus is visible on the bone making up of the sockets of the molar and incisor teeth.

3 and 4. Ground section and contact autograph of animal sacrificed 2 hours after injection. No appreciable change can be seen.

5 and 6. Corresponding preparations of animal sacrificed 2 days after injection. There is an increased reaction of the 3rd molar which has not erupted. The 3rd molar also shows an enamel line. The incisor tooth shows the beginning of an enamel reaction on its inferior surface.

7 and 8. Corresponding preparations of animals sacrificed 8 days after injection. The animal reaction has elongated. Otherwise there is no change from the other autographs.



PLATE 2

Explanation of Figures

Coated autographs and ground sections of the lower jaws of adult rats sacrificed at 5 minutes, 2 hours and 8 days after injection of  $P^{32}$ . There is very little change in the site of the  $P^{32}$  distribution during the 8 days.

1 and 2. Ground section of lower jaw and contact autograph of animal sacrificed 5 minutes after injection. The most intense reaction is visible on the inner side of the dentin of the incisor tooth which is still growing. There is no reaction of the dentin of the molar teeth. The alveolar bone making up the tooth sockets also reacts intensely.

3 and 4. Similar preparations of animal sacrificed 2 hours after injection. There is no recognizable change in the deposition of  $P^{32}$ .

5 and 6. Similar preparations of animals sacrificed 8 days after injection. The dentin of the incisor tooth still shows the greatest reaction. The dentin of the molar tooth here shows a minimal reaction. The enamel has not reacted at any time interval.

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PLATE 3

Explanation of Figures

Histochemical reaction of the tooth in the newborn rat.

1. Masson stain showing the enamel covering only one side of the molar tooth.

2. Von Kossa stain. This was done by staining very lightly. Under these conditions, the dentin appears black. So does the bone seen on the left-hand side. The thinner band of enamel is only moderately stained and appears grey. It is limited on the outside by a very thin membrane described as the supra-ameloblastic membrane, which stains more intensely than the enamel itself.

3. Radioautograph at 24 hours after injection of  $P^{32}$ . A faint dark band is visible in the dentin. A slight reaction occurs throughout the enamel. The supra-ameloblastic membrane shows a reaction, at least in its upper and lower portions.



Plate 1

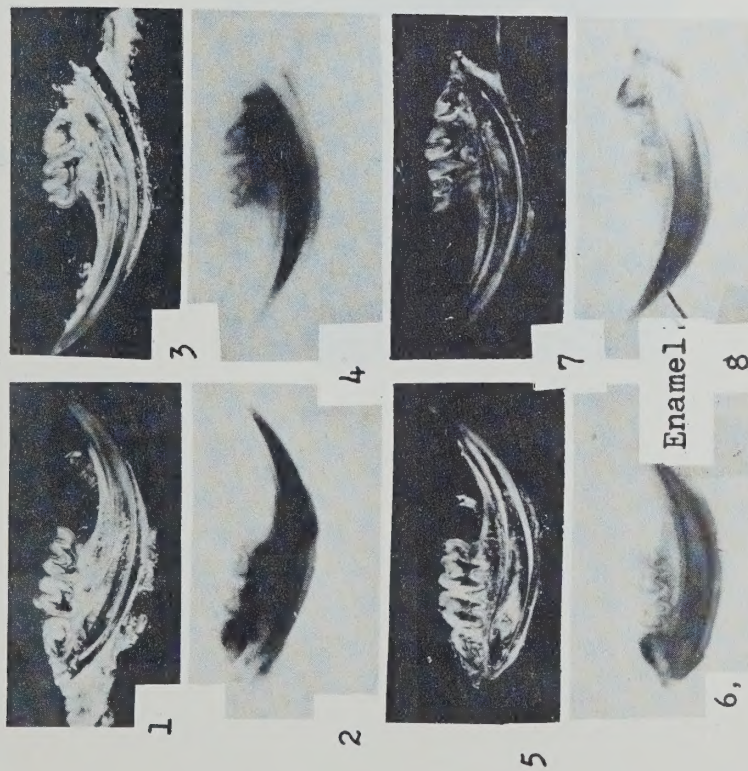


Plate 2

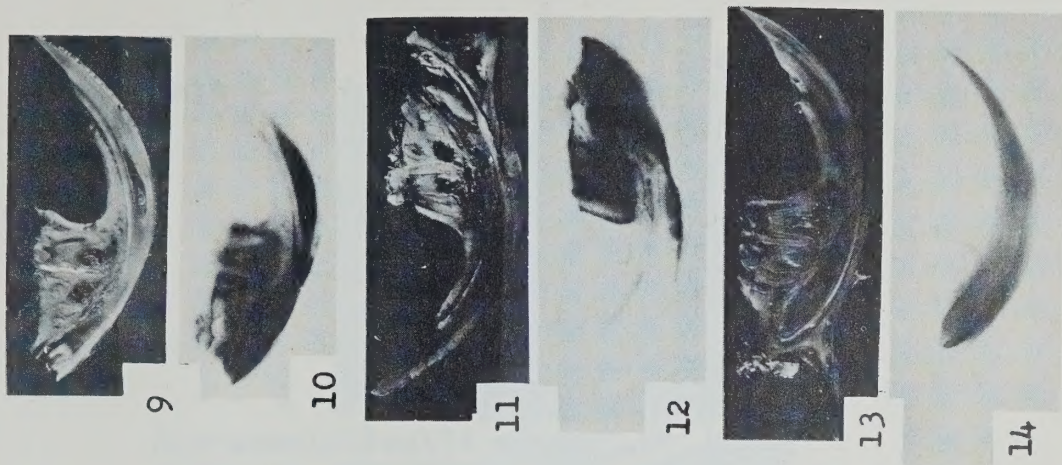
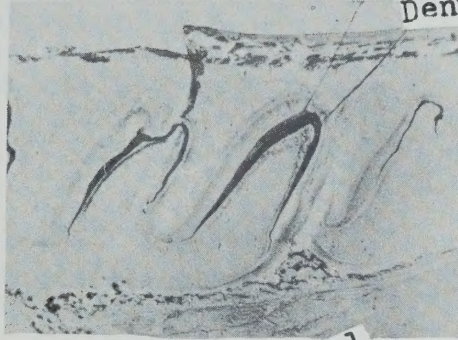






Plate 3

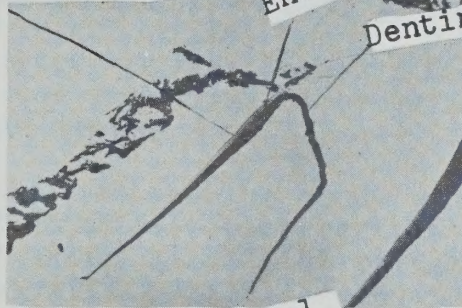
Enamel  
Dentin



1

Supraameloblastic  
membrane

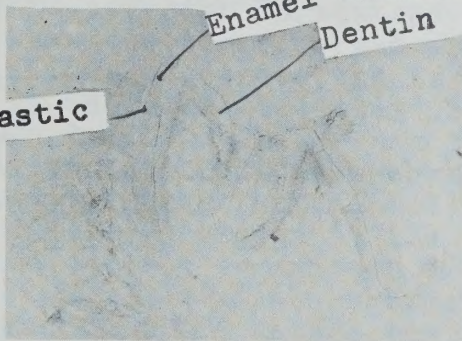
Enamel  
Dentin



2

Enamel  
Dentin

Supraameloblastic  
membrane



3





## APPENDIX "P"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1951.

Subject: A histological study of tooth germ development both intra- and extra-alveolar with a survey of pathological changes in the developing tooth.

Grantees: Dr. J. W. Abbiss and Dr. A.G. Nutlay

Project: DR 27 Amount of Grant: \$200.

#### SUMMARY OF WORK

This summary consists of excerpts from the final paper which was outlined in the last report.

The grantees intend to confine their study in the first place to problems related to the enamel only. They propose to approach the subject by a study of the normal embryology of the tooth and a comparison of the structure and histology of intra-alveolar and extra-alveolar teeth.

It is hoped that the comparative study will show differences between intra-alveolar and extra-alveolar teeth which can be evaluated. The enamel organ is unique among epithelial structures in that it forms a hard, calcified material (enamel). In comparing the intra-alveolar ("normal") and extra-alveolar formations (for example in teratomata) it is obvious that the enamel organ is in a different environment as regards relations to stress and to extraneous influences (organisers). It is generally taken that the ameloblasts is the prime factor in tooth development in that it is held to provide a specific stimulus to the local mesenchyme so that it becomes dentinogenic and one presumes that the dental follicle is a further reaction to the original stimulus produced by the enamel. Clearly, however, two factors operate, namely organisers and, after birth, reactions to stress. While what one might consider that the prime stimulus (or organiser) comes from the enamel the question of specific organisers in the dentinogenic tissues and the importance of the stress factor have not been evaluated. One has good reason to hope that a careful study of development of normal teeth before and after birth, before and after stress, and a comparative study between intra-alveolar and extra-alveolar teeth may yield important clues; since 1) stress does not operate in certain extra-alveolar teeth e.g. in teratomata and since 2) the external environment in extra-alveolar teeth may be "abnormal" and therefore any external organisers may be presumed to function differently from external organisers in the alveoli.

It is generally accepted that the ameloblast provide a specific stimulus to the local mesenchyme so that it becomes dentinogenic and only when this is accomplished the ameloblasts form enamel and it should be remembered that it is still undecided



whether ameloblasts form enamel intra-cellular or in the cell surface.

In an extra-alveolar tooth development one should be able to settle the action of surrounding (non specific) mesenchyme on specific formations (enamel organ); or whether the enamel organ alone acts on specific mesenchyme tissues and thus making the formation of odontoblasts dependent on the enamel organ. There is ample opportunity to debate the specific character of the dental follicle. This is an important point since the mesenchyme (dental) follicle forms the following structures: 1) cementum, 2) periodontal membranes, 3) alveolar process.

The problem of decalcifying teeth has been brought up in the interim report. Since that time no substantial changes toward an improvement have been reported in the literature of pathologic laboratory technique. The grantees, after solving considerable problems, are using the technique of Gelfand et al. in decalcifying bones and teeth. This is particularly important in the view of the study which they undertook. The organic elements of the enamel (very scarce) must be studied without the harmful influence of the decalcifying agents. There are many points of interest connected with this promising method of technique.

The non-decalcified teeth are studied by polarised light. The new X-ray diffraction apparatus which the grantees are constructing with the help of the Institutes of Pathology in Halifax, will analyse the consistence and reveal the position of submicroscopic elements of the enamel.

The technique of decalcifying bones and teeth presently used by the grantees is described by Richman, Gelfand and Hill in Archives of Pathology, Vol. 44, page 92, 1947.

"A. G. Nutlay"

Signature

Date: February 10, 1951.



## APPENDIX "Q"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1951

Subject: The effects of the physical consistency of food on the periodontium of the rat.

Grantee: Chas. H. M. Williams

Project No. DR 24

Amount of Grant: \$1735.

#### SUMMARY OF WORK

This report is presented in two sections. The main portion, the bound copy, is a detailed description of all procedures and data developed during the study of the first two groups of animals employed in the study. This second portion of the report is a very brief summary of procedures and observations concerning both the above-mentioned two groups of animals and the third and final group.

Previous reports concerning this study have related to observations on two groups of animals. In the first study sixty white rats were divided into two groups at date of weaning. One group was fed hard Fox Breeder cubes plus water and the other group was fed the same food but after it had been ground to a flour-like texture and then mixed with water to a pappy consistency.

#### Main Observations on First Group

The mandibles of the hard diet group were significantly larger by planimetric measurement, by volume and by bucco-lingual thickness than those of the soft diet group.

There was no significant difference in the density of the bone of the mandibles of the two groups.

The gingival tissues of the hard diet group exhibited epithelium with cells in dense and orderly arrangement and a heavy continuous protective keratin layer. There was very little round cell infiltration of the connective tissue corium of the gingival papillae.

The epithelium of the gingival tissues of the soft diet group exhibited a loose polyhedral arrangement of the cells and a less dense protective keratin layer. There was frequent round cell infiltration of the corium of the gingival tissue.

The second phase of the study involved a repetition of the feeding procedure used for the first group of animals. But the animals employed were adults which, at the beginning of the study, were about nine months of age. They had been fed hard Fox Breeder cubes since the date of weaning. Forty animals were employed.



One half of the group continued to eat the hard food and the other half received the soft form of the same food. The period of observation was four months.

#### Main Observations on Second Group

Essentially the same differences in area, volume and density of the mandibles and of the conditions of the gingival tissues were noted as have been reported above concerning the younger animals. It is still not known whether the mandibles of the hard diet animals continued to increase in bulk throughout adult life, or whether those which were switched to soft food after attaining adult development had atrophy of the mandibles. A comparison of the material from this group with that about to be referred to below should yield information on this point.

The animals in both of the above mentioned groups were under observation for periods of four months. In order to gain information concerning a longer term effect of hard and soft food a study involving eighty rats and an experimental period of a full calendar year was instituted. The animals were placed, at date of weaning, on the same feeding programme as described for the other groups.

The animals of this last study have been sacrificed and all of the material to be examined macroscopically has been prepared. It is being studied for the preparation of a report. The material to be examined microscopically is in the course of preparation. It is contemplated that the activities of this project will be essentially completed within the next three months.

When the data has been organized for presentation, a copy of the report will be sent to your committee.

Information gained from this study appears to have added significantly to the knowledge concerning the physiology of dentitions. Dr. D.G. Watt, my assistant, and myself wish to tender our thanks to the Associate Committee on Dental Research of the National Research Council for the support which allowed the completion of this phase of a large study.

Date: February 27, 1951.

"C.H.M. Williams"

Signature



## APPENDIX "R"

### Suggested Research Projects (Min.4(x), 13th Mtg.)

By decision of the Committee, projects suggested as research topics are listed hereunder for consideration by Committee members:

| <u>Proposed Subject</u>                                                                                                                                      | <u>Suggested by:</u>                                                                    | <u>Date</u> |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------|
| 1. Gold inlays over amalgams                                                                                                                                 | Dr. M.H. Garvin                                                                         | Oct. 1950   |
| Little information is available as to whether gold inlays over amalgams cause disintegration of the cement.                                                  |                                                                                         |             |
| 2. A study of root canals of teeth                                                                                                                           | Dr. M. H. Garvin                                                                        | Oct. 1950   |
| This is a very practical research subject as the results would be of value to every practitioner.                                                            |                                                                                         |             |
| Changes in the Calcium and Phosphate Concentrations of Saliva and Inorganic Salt Solutions on Shaking with Calcium Phosphate,<br>by J. J. RAE and G.T. CLARK | J. Dent. Res., 27: 34-37. 1948.                                                         |             |
| An Improved Method for Processing Acrylic Dentures,<br>by D.R. CHRISTIE                                                                                      | J. Can. Dent. Assoc., June, 1948. 15 pp. (App. "B", 8th Mtg., A.C.D.S., 26 Oct., 1948). |             |
| The Therapeutic Properties of Periodontal Cement Packs,<br>by W.J. LINGHORN and D.C. O'CONNELL                                                               | J. Can. Dent. Assoc., April, 1949. 7 pp.                                                |             |
| Phosphatase in Saliva,<br>by J.T. DENTON and J.J. RAE                                                                                                        | J. Dent. Res., Feb. 1949. 4 pp.                                                         |             |
| The Treatment of Acute Oral Vincent's Infection,<br>by W.J. LINGHORN and D.F. SYEDAN                                                                         | J. Can. Dent. Assoc., July, 1949. 4 pp. (D.C.S. No. 1949).                              |             |

March, 1951.





# APPENDIX "S"

## ASSOCIATE COMMITTEE ON DENTAL RESEARCH

### PAPERS PUBLISHED

| Committee<br>Paper No. | Title and Author                                                                                                                                             | Reference                                                                                                                 |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| 1                      | New Aspects of Periodontal Research,<br>by H. K. BOX                                                                                                         | Presented at National Convention of Canadian Dentists, Toronto, 29 May 1946. (App. "C", 5th Mtg. A.C.D.R., 25 Feb. 1947). |
| 2                      | Concerning the Pathogenesis of Periodontal Disease,<br>by H. K. BOX                                                                                          | J. Periodontology, 19: 11-20. 1948. (App. "G", 7th Mtg., A.C.D.R., 2 Mar. 1948).                                          |
| 3                      | The Effect of Various Inorganic Salts on the Solubility of Calcium Phosphate, Tooth Enamel and Whole Teeth in Lactic Acid,<br>by J. J. RAE and C.T. CLEGG    | J. Dent. Res. 27: 52-53. 1948.                                                                                            |
| 4                      | Changes in the Calcium and Phosphate Concentrations of Saliva and Inorganic Salt Solutions on Shaking with Calcium Phosphate,<br>by J. J. RAE and C.T. CLEGG | J. Dent. Res. 27: 54-57. 1948.                                                                                            |
| 5                      | An Improved Method for Processing Acrylic Dentures,<br>by D.R. CHRISTIE                                                                                      | J. Can. Dent. Assoc., June, 1948. 15 pp. (App. "R", 8th Mtg., A.C.D.R., 26 Oct., 1948).                                   |
| 6                      | The Therapeutic Properties of Periodontal Cement Packs,<br>by W.J. LINGHORNE and D.C. O'CONNELL                                                              | J. Can. Dent. Assoc., April, 1949. 7 pp.                                                                                  |
| 7                      | Phosphatase in Saliva,<br>by J.T. DENTAY and J.J. RAE                                                                                                        | J. Dent. Res., Feb. 1949. 4 pp.                                                                                           |
| 8                      | The Treatment of Acute Oral Vincent's Infection,<br>by W.J. LINGHORNE and D.F. STEDMAN                                                                       | J. Can. Dent. Assoc., July, 1949. 6 pp. (N.R.C. No. 1699).                                                                |





| <u>Committee<br/>Paper No.</u> | <u>Title and Author</u>                                                                                                                                           | <u>Reference</u>                                      |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| 9                              | A Comparison of Nine Local<br>Anaesthetics, by<br>H.S. HAMILTON, B.A. WESTFALL<br>and J.K.W. FERGUSON                                                             | J. Pharmacol. Expt.<br>Therap. 94: 299-<br>307. 1948. |
| 10                             | New Methods of Repairing<br>Acrylic Dentures without<br>Distortion, by<br>D.R. CHRISTIE                                                                           | J. Can. Dent. Assoc.,<br>Nov. 1949. 11 pp.            |
| 11                             | The Corrosion of Cobalt<br>Chromium Alloys in Commercial<br>Cleaning Agents, by<br>D.E.R. COGGLES, O.J. WALKER<br>and H.R. MACLEAN                                | J. Can. Dent. Assoc.<br>16: 75-82. 1950.              |
| 12                             | The Relation between Buffering<br>Capacity, Viscosity and Lacto-<br>bacillus Count of Saliva, by<br>J.J. RAE and C.T. CLEGG                                       | J. Dent. Res. 28:<br>589-593. 1949.                   |
| 13                             | Mineralization of the Growing<br>Tooth as Shown by Radio-<br>Phosphorus Autographs (17692),<br>by L.F. BELANGER and C.P. LEBLOND                                  | Proc. Soc. Expt.<br>Biol. Med. 73: 390-<br>391. 1950. |
| 14                             | Radio-autographic Visualization<br>of Bone Formation in the Rat,<br>by C.P. LEBLOND, G.W. WILKINSON,<br>L.F. BELANGER and J. ROBICHON                             | Am. J. Anat. 86: 2.<br>1950.                          |
| 15                             | Fluoride Studies Related to the<br>Human Diet, by<br>MARY P. HAM and M. DOREEN SMITH                                                                              | Can. J. Res. F,<br>28: 227-233. 1950.                 |
| 16                             | Studies in the Regeneration and<br>Reattachment of Supporting<br>Structures of the Teeth.<br>I. Soft Tissue Reattachment, by<br>W.J. LINGHORNE and D.C. O'CONNELL | J. Dent. Res. 29:<br>419-428. 1950.                   |
| 17                             | An Examination of the Fluoride<br>Content of Teeth from some Ont-<br>ario Districts, by<br>SHIRLEY R. HARNESS and M. DOREEN<br>SMITH                              | J. Can. Dent. Assoc.,<br>Jan. 1951. 5 pp.             |

The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

March, 1951.





Members of the Associate Committee on Dental Research  
and its Executive, with their terms of appointment

|                                                                                                                 | <u>Term to Expire</u> |
|-----------------------------------------------------------------------------------------------------------------|-----------------------|
| * 1. Dr. R. G. Ellis, Chairman,<br>Dean of the Faculty of Dentistry,<br>University of Toronto,<br>Toronto, Ont. | 31 March, 1951        |
| 2. Dr. C. J. Mackenzie, (ex officio)<br>President,<br>National Research Council,<br>Ottawa, Ont.                | -                     |

Representing the Dental Profession

|                                                                                                                                 |                |
|---------------------------------------------------------------------------------------------------------------------------------|----------------|
| 3. Dr. J. S. Bagnall,<br>Dean, Faculty of Dentistry,<br>Dalhousie University,<br>Halifax, N.S.                                  | 31 March, 1952 |
| * 4. Dr. E. Charron,<br>Dean of the Faculty of Dental Surgery,<br>University of Montreal,<br>Montreal, Que.                     | 31 March, 1951 |
| 5. Dr. M. H. Garvin,<br>Editor-in-Chief,<br>Journal of the Canadian Dental Assoc.,<br>314 Somerset Building,<br>Winnipeg, Man.  | 31 March, 1953 |
| 6. Dr. H. R. MacLean,<br>Lecturer in Operative Dentistry,<br>Faculty of Dentistry,<br>University of Alberta,<br>Edmonton, Alta. | 31 March, 1953 |
| 7. Dr. R. H. McDougall,<br>1505 Hampshire Road,<br>Victoria, B.C.                                                               | 31 March, 1952 |

Representing the Royal Canadian Dental Corps

|                                                                                                                                                               |   |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| * 8. Col. E. M. Wansbrough, (ex officio)<br>Director General of Dental Services<br>Royal Canadian Dental Corps,<br>Dept. of National Defence,<br>Ottawa, Ont. | - |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|---|





17. Representing the Division of Dental Health  
Department of National Health and Welfare

31 March, 1953

9. Dr. H. K. Brown, (ex officio)  
Chief, Dental Health Division,  
Dept. of National Health and Welfare,  
Ottawa, Ont.

-

Representing the Basic and Medical Sciences

10. Dr. C. H. Best,  
Director, Banting and Best Dept. of  
Medical Research,  
University of Toronto,  
Toronto, Ont. 31 March, 1953
11. Dr. J. B. Collip,  
Dean of the Faculty of Medicine,  
University of Western Ontario,  
London, Ont. 31 March, 1952
12. Dr. O. W. Ellis,  
Dept. of Metallurgy,  
Ontario Research Foundation,  
Toronto, Ont. 31 March, 1951
13. Dr. J.K.W. Ferguson,  
Head of the Dept. of Pharmacy  
and Pharmacology,  
University of Toronto,  
Toronto, Ont. 31 March, 1951
14. Dr. A. W. Ham,  
Prof. of Anatomy,  
Faculty of Medicine,  
University of Toronto,  
Toronto, Ont. 31 March, 1952
15. Dr. J. B. Macdonald,  
Assistant Prof. of Bacteriology,  
Faculty of Dentistry,  
University of Toronto,  
Toronto, Ont. 31 March, 1951
16. Dr. J. J. Rae,  
Dept. of Chemistry,  
University of Toronto,  
Toronto, Ont. 31 March, 1953





- \* 17. Dr. D. L. Thomson, 31 March, 1953  
Chairman of the Department of  
Biochemistry,  
McGill University,  
Montreal, Que.
- \* 18. Mr. S. J. Cook, Secretary  
Public Relations Branch,  
National Research Council,  
Ottawa, Ont.

\* Members of the Executive

7. Dr. G. H. Ellis  
8. Dr. J. S. W. Ferguson  
9. Dr. M. H. Garvin

10. Dr. A. W. Dowd  
11. Dr. J. W. Garrod  
12. Dr. G. A. Macleod  
13. Dr. A. A. Martin  
14. Dr. W. S. Macleod  
15. Dr. J. S. Macleod  
16. Dr. J. L. Macleod  
17. Dr. J. S. Macleod  
18. Dr. J. S. Macleod

(11) To other persons:-

- 19-20. Deans of Dental Schools (who are not members of the Council)  
19. Alberta - Dr. W. S. Macleod  
20. McGill - Dr. W. S. Macleod  
21. Austin Copy (General Secretary)  
22. Austin Copy  
23. Library  
24-25. Records





INITIAL DISTRIBUTION

(i) To members of the

Associate Committee on Dental Research

- |                                     |                                      |
|-------------------------------------|--------------------------------------|
| 1. Dr. R. G. Ellis, <u>Chairman</u> | 10. Dr. A. W. Ham                    |
| 2. Dr. C. H. Best                   | 11. Dr. J. B. Macdonald              |
| 3. Dr. J. S. Bagnall                | 12. Dr. C. J. Mackenzie              |
| 4. Dr. H. K. Brown                  | 13. Dr. H. R. MacLean                |
| 5. Dr. E. Charron                   | 14. Dr. R. H. McDougall              |
| 6. Dr. J. B. Collip                 | 15. Dr. J. J. Rae                    |
| 7. Dr. O. W. Ellis                  | 16. Dr. D. L. Thomson                |
| 8. Dr. J.K.W. Ferguson              | 17. Col.E. M. Wansbrough             |
| 9. Dr. M. H. Garvin                 | 18. Mr. S. J. Cook, <u>Secretary</u> |

(ii) To other persons:-

19-20 Deans of Dental Schools (who are not members of the Committee)

19. Alberta - Dr. W. S. Hamilton

20. McGill - Dr. D. Prescott Mowry

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UNIVERSITY OF TORONTO  
FACULTY OF DENTISTRY  
OFFICE OF THE DEAN

REC'D. MAY 4 - 1951

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REFERRED TO \_\_\_\_\_  
FILE \_\_\_\_\_



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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS  
OF THE  
FOURTEENTH MEETING  
OF THE  
ASSOCIATE COMMITTEE ON  
DENTAL RESEARCH

OTTAWA

27 SEPTEMBER 1951







## TABLE OF CONTENTS

|                                                       | <u>Page</u> |
|-------------------------------------------------------|-------------|
| 1. Attendance .....                                   | 1           |
| 2. Chairman's Remarks .....                           | 1           |
| 3. Minutes of the 13th Meeting .....                  | 2           |
| 4. Business Arising out of the Minutes .....          | 2           |
| 5. DR 1 - Dr. J.J. Rae .....                          | 3           |
| 6. DR 12 and Dr 25 - Dr. A.E.R. Westman .....         | 3           |
| 7. DR 13 - Dr. M. Doreen Smith .....                  | 4           |
| 8. DR 22 - Dr. C.H. Best .....                        | 6           |
| 9. DR 23 - Dr. C.P. Leblond .....                     | 6           |
| 10. DR 24 - Dr. C.H.M. Williams .....                 | 6           |
| 11. DR 28 - Dr. O.J. Walker .....                     | 7           |
| 12. DR 33 - Dr. H.A. Hunter .....                     | 7           |
| 13. DR 34 - Dr. J.P. Lussier .....                    | 8           |
| 14. DR 35 - Dr. J.B. Macdonald .....                  | 8           |
| 15. DR 36 - Dr. G. Nikiforuk .....                    | 8           |
| 16. DR 37 - Dr. R.E. Moyers .....                     | 9           |
| 17. DR 38 - Dr. K.J. Paynter .....                    | 9           |
| 18. DR 39 - Dr. A.G. Racey .....                      | 9           |
| 19. Fellowships .....                                 | 9           |
| 20. Finances .....                                    | 11          |
| 21. Estimates, 1952-53 .....                          | 11          |
| 22. Applications for Grants, 1951-52 .....            | 11          |
| 23. Dr. Bagnall's Bibliography on Caries Research ... | 13          |
| 24. Air Travel (1951-52) .....                        | 14          |
| 25. Travel to Scientific Meetings .....               | 14          |
| 26. Visitors at Next Meeting .....                    | 14          |
| 27. New Projects .....                                | 14          |





TABLE OF CONTENTS

|                                | <u>Page</u> |
|--------------------------------|-------------|
| 28. Date of Next Meeting ..... | 15          |

Appendices

|              |                                                                                                                                                                          |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Appendix "A" | Investigations of materials for use as cements for methacrylate inlays and of methods for use of methacrylate as direct dental fillings, DR 12 and DR 25                 |
| Appendix "B" | The effect of thiouracil on the development of molar teeth of rats, DR 38                                                                                                |
| Appendix "C" | Demineralization of hard tissues as bone and teeth by organic chelating agents, DR 36                                                                                    |
| Appendix "D" | Studies of calcium metabolism in tooth with the aid of $Ca^{45}$ , DR 34                                                                                                 |
| Appendix "E" | Sectioning of teeth and surrounding structures, DR 39                                                                                                                    |
| Appendix "F" | The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure, DR 33                                              |
| Appendix "G" | Chemical mechanisms in dental caries, DR 1                                                                                                                               |
| Appendix "H" | Part I - An investigation of the mechanism of repair of the supporting structures of the teeth; Part II - Fusospirochetal infection and pre-existing inflammation, DR 22 |
| Appendix "I" | Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radiocarbon, DR 23                                                      |
| Appendix "J" | Experimental fusospirochetal infection with mixtures of pure cultures, DR 35                                                                                             |
| Appendix "K" | Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to pabulum history of the children's diets, DR 13                               |
| Appendix "L" | Development of a method for determining fluorine by means of a photo-electric colorimeter, DR 28                                                                         |
| Appendix "M" | Papers Published, Associate Committee on Dental Research.                                                                                                                |
| Appendix "N" | A Study of Migration of Tooth Germs During and Before Mixed Dentition. (Fellowship Grant)                                                                                |
| Appendix "O" | Oral Pathology and Bacteriology (Fellowship Grant)                                                                                                                       |
| Appendix "P" | Suggested Research Projects (Min.4(x)13th Mtg.)and (Min. 27, 14th Mtg.)                                                                                                  |





NATIONAL RESEARCH COUNCIL

Proceedings

Fourteenth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Council Chamber, National Research Building,  
9:30 a.m., 27 September, 1951.

1. Attendance

Dr. R. G. Ellis, Chairman  
Dr. J. S. Bagnall  
Dr. H. K. Brown  
Dr. A. C. Burton  
Dr. J. B. Collip  
Dr. E. Charron  
Dr. M. H. Garvin  
Dr. L. A. Kilburn  
Dr. J. B. MacDonald  
Dr. H. R. MacLean  
Dr. R. A. McDougall  
Dr. J. J. Rae  
Dr. D. L. Thomson

Mr. S. J. Cook, Secretary

By invitation

Dr. C. P. Leblond  
Dr. M. D. Smith

Apologies for their absence were received from - Dr. C. H. Best, Dr. A. W. Ham, (who was on a tour of Western Cancer Clinics), Dr. J. B. Hamilton, and Brig. E. M. Wansbrough, who was attending The Defence Dental Association's Annual Meeting.

Grantees who had been invited to report at this meeting but who were unable to do so included - Dr. W. J. Linghorne, Dr. H. A. Hunter, and Dr. C. H. M. Williams.

2. Chairman's Remarks

THE CHAIRMAN welcomed Dr. A. C. Burton and Dr. L. A. Kilburn both of whom were attending their first meeting. He expressed regret that Dr. Hamilton also a new member had been unable to come because of College duties.





On behalf of the Committee the Chairman offered his congratulations to Dean Charron and Dean Bagnall both of whom had recently been honoured by being made Fellows in Dental Surgery of the Royal College of Dental Surgeons of Edinburgh. He also extended the best wishes of the Committee to the Secretary on his recent marriage.

### 3. Minutes of the 13th Meeting

It was MOVED by Dr. Bagnall and SECONDED by Dr. MacLean,

That the Minutes of the 13th Meeting having been circulated be taken as read and approved. CARRIED.

### 4. Business Arising out of the Minutes

#### (i) DR 15 (Min. 9, 13th Mtg.)

A reply from Dr. Norma Ford Walker to a letter written by the Secretary contained the information that the thesis on this project is still in preparation.

#### (ii) DR 32 (Min. 20, 13th Mtg.)

A letter from Dr. Ham stated that it had not been possible to complete the report which had been promised for the Autumn meeting but he hoped to be able to make a full report at the Spring meeting of the Committee.

#### (iii) DR 22 (Min. 32, 13th Mtg.)

THE SECRETARY reported that by letter ballot of the Executive the grant made to Dr. Best of \$2300 was increased by \$875 making a total of \$3175. This is for part 1 of Dr. Best's investigation which relates to the repair of the supporting structures of teeth.

By another letter ballot a grant of \$1350 was made for work under part II of this investigation dealing with fusospirochetal infection. A final report for the period ending 30 June, 1951, was submitted.

#### (iv) DR 39 Dr. A.G. Racey - Sectioning of teeth and surrounding structures. (Min. 32, 13th Mtg.)

By letter ballot dated 12 April, 1951, a grant of \$1600 was made to Dr. Racey. Through an unfortunate oversight this application was missed during the consideration of applications at the March meeting of the Committee; subsequently, at the request of the Chairman a précis was sent to the members of the Executive with a letter ballot and the application was then approved.

THE CHAIRMAN reported briefly on these two projects and drew attention to the list of papers that had arisen from this work.





(v) DR 31 (Min. 32, 13th Mtg.)

The application by Dr. L.B. Jaques and Mr. G.J. Millar for \$2,000 which was deferred at the March meeting was submitted to a letter ballot of the Executive together with some explanatory information obtained by the Chairman. As the result of the ballot the application for a grant was not approved, but on receipt of a letter from the grantee pointing out that certain expenditures had already been incurred, the Chairman authorized the Secretary to offer the grantee compensation in the amount expended and as a result \$188 was advanced to him.

Consideration of Reports from Grantees

5. DR 1 - Dr. J.J. Rae

"Chemical Mechanisms in Dental Caries"

DR. RAE presented a summary and interim report No. 12 on his project both of which appear in APPENDIX "G"

THE CHAIRMAN said it was very interesting that out of this project Dr. Rae has been able to question the value of ammonia in dentifrices.

DR. MACDONALD observed that penicillin is now being used in some dentifrices. The Research Committee of the Canadian Dental Association is reviewing the literature on this subject and has found one report which shows that some reduction of caries was obtained by the use of 500 units of penicillin. This was a school project carried on for two years with good controls. A reduction of 54% in dental caries was noted.

A second study carried on in an orphanage without controls showed no significant difference. Hence the use of penicillin by the general public does not seem to be advantageous. It was suggested that some persons may be sensitive to penicillin. Development of resistance was not noticed. Similarly when sulphonamides are used in control of upper respiratory infections there is at first some reduction but gradually the system becomes used to the product and the beneficial effects in later treatment are of less importance.

6. DR 12 and DR 25 - Dr. A.E.R. Westman

"Investigations of Materials for use as Cements for Methacrylate Inlays and of Methods for the use of Methyl Methacrylate as Direct Dental Fillings"

Both of these projects were terminated as of 30 June 1951 and the unexpended portion of the grant was made available for other projects of the Committee. The summary submitted by the grantee appears in APPENDIX "A"

THE CHAIRMAN reported briefly on these two projects and drew attention to the list of papers that had arisen from this work.





DR. BURTON inquired whether there was any way in which the Committee could facilitate the use of the findings developed by the Ontario Research Foundation under this project.

THE CHAIRMAN replied that the work had all been published in the Journal of the Canadian Dental Association so that the results had been brought to the attention of all practicing dentists in Canada. He said that Mr. Christie, who had carried on the work, had also given numerous addresses before interested groups concerning the new techniques. It was outside the scope of the Committee itself to undertake the promotion of new dental materials.

DR. GARVIN took the opportunity of drawing the attention of the members to the fact that the Canadian Dental Association had passed a resolution supporting the publication of Canadian research results in the Journal of the Canadian Dental Association. He thought that some of Dr. Rae's work might be of interest to readers if published in summary form in the Journal of the Canadian Dental Association.

DR. RAE said that he usually published his results in the Journal of Dental Research. He said however that he could easily prepare notes on his work for publication in the Journal of the Canadian Dental Association if this were desired.

THE CHAIRMAN, concluding this discussion on DR 12 and 25, said that as the Ontario Research Foundation has no other man who can take up the work that Mr. Christie has been doing the project will be dropped for the present.

#### 7. DR 13 - Dr. M. Doreen Smith

"Fluoride Content of Enamel and Dentin of Teeth from Toronto Children Studied in Relation to Pabulum History of the Children's Diets"

A summary and a report were presented by Dr. M. Doreen Smith. These appear in APPENDIX "K"

THE CHAIRMAN asked Dr. Smith also to report on the present situation on DR 29 which project is now out of the hands of the Committee but is being carried on without financial support from the Council in a study for a Ph.D thesis.

DR. SMITH said that satisfactory progress was being made and that a report would be available in the Spring. Some analytical difficulties had been experienced in connection with the analyses of faeces for fluoride content.

Regarding the financing of DR 13, she said that it would be useful if some money could be allocated under the grant for the purchase of teeth; she had found that the saving and cleaning of teeth by dentists' assistants could only be secured if some compensation were provided.







DR. BROWN said that his Department also had to pay for teeth in order to secure case histories. He thought it was quite fair to offer compensation to dentists' assistants who undertook the collection of teeth for research purposes.

THE CHAIRMAN said that this subject might be discussed under new grants.

DR. BAGNALL asked whether it was possible to distinguish among the Brantford teeth, as to the length of time of exposure to fluorine.

DR. BROWN said that teeth used were only from children born in Sarnia and Brantford and that both the date of extraction and the age of the child were recorded. In Stratford 1.3 p.p.m. F is obtained from wells and food may add a little to this percentage but in Sarnia the fluorine content of water and food is negligible.

THE CHAIRMAN said that no significant differences were observed between the children fed on pabulum and those fed on non-pabulum foods, but that differences in the fluorine content of water supplies appear to have a pronounced effect.

DR. RAE wanted to know why the fluorine content of dentine is higher than that in the enamel.

THE CHAIRMAN said that the enamel was less likely to be affected by food than the dentine would be.

DR. BROWN added that in the Brantford studies the enamel on children's teeth had been completed before the practice of adding fluorine to the drinking water was adopted and yet there had been a considerable reduction in the incidence of caries. It would appear therefore that the topical application of fluorine had some effect.

DR. MACDONALD pointed out that the literature shows enamel is not so inert as had been supposed. Fosdick reported that in some experiments it had been possible to introduce fluorine into enamel by an osmotic effect.

DR. THOMSON remarked that very little information is available from the report on the dentine-enamel ratio. It appears to depend on the method of making the calculation.

DR. BURTON added that the average of ratios is good but the ratio of two averages has no meaning.

DR. SMITH said, in reply to a question by Dr. Rae, that the results from DR 29 are to be submitted for publication in the Journal of Nutrition.

DR. BURTON hoped that where reports are given to the Committee, a clue will be provided as to the difference in means and it was AGREED that this should be done in all reports.





8. DR 22 - Dr. C.H. Best

"Part I - An investigation of the mechanism of repair of the supporting structures of the teeth;

"Part II - Fusospirochetal infection and pre-existing inflammation"

A summary and a report on Part I together with a final report on Part II for the period ending 30 June 1951 appear in APPENDIX "H"

THE CHAIRMAN said that it had been hoped Dr. Linghorne would be present to report on Part I but owing to illness this had not been possible. He thought Dr. Linghorne might be asked to come to the next meeting.

As regards Part II he had already pointed out that this project had been discontinued in June 1951. Three papers have been published on work done under this project.

DR. MACDONALD said that it costs \$300 to maintain the refrigeration with dry ice using a 1½ to 2 cu. ft. refrigerator. He had been informed that the deep freeze people estimated that similar refrigeration with their equipment would cost \$3500.

DR. BURTON said that a cold chamber should be cheaper.

DR. COLLIP suggested that useful information on this subject might be obtained from Dr. Parks who has developed a means for the preservation of blood at temperatures down to -78°C.

Dr. Parks is at the Medical Research Council Laboratory, Mill Hill, London, England.

9. DR 23 - Dr. C.P. Leblond

"Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radiocarbon"

DR. LEBLOND presented his report which appears in APPENDIX "I" He also made use of the blackboard and slides to give the members a clear and excellent presentation of the work he is doing under this project.

THE CHAIRMAN thanked Dr. Leblond most cordially for his report.

10. DR 24 - Dr. C.H.M. Williams

"The effects of the physical consistency of food on the periodontium of the rat"

THE CHAIRMAN said that he had extended an invitation from the Committee to Dr. Williams to present in person his report under DR 24 which was laid on the table at the last meeting of the Committee.





Dr. Williams had been unable to come but had written to the Secretary under date of 24 September as follows:-

" At the time of the last report it was mentioned that groups of animals on hard and soft forms of the same food had been fed over a one-year period. It was our hope to compare the influences of a full-year experimental period against the four-month experimental period previously reported. The total weights of the pairs of animals in the previous groups was almost exactly the same and we were therefore able to compare the weights and bulk of the mandibles to determine the influences of hard and soft foods. In the full year group an obvious variation in total weights of the animals exists and the comparison of their mandibles alone on the basis of weight, volume, etc. would probably be open to error.

" It is our opinion that the variations in total weights of the pairs of animals is a result of irregular feeding. During the last four months of the experimental period a technician, who has since been discharged, was responsible for the feeding. We are extremely disappointed that the material from this group may not be employed because the results arising from it had the possibility of contributing to the knowledge concerning the physiologic evolution of dentitions as it is affected by vigorous or indolent function."

THE SECRETARY was directed to reply to Dr. Williams thanking him for his letter of 24 September and saying that it would be added to the record of project DR 24. The Secretary was also asked to express the regret of the Committee that Dr. Williams had not found it possible to be present at the meeting to discuss his report in person.

11. DR 28 - Dr. O.J. Walker

"Development of a method for determining fluorine by means of a photo-electric colorimeter"

DR. MACLEAN presented this report at the request of the Chairman. The summary prepared by Dr. Walker appears in

APPENDIX "L"

DR. RAE said that he would like to know where the papers referred to in the report are to be published.

THE SECRETARY promised to obtain this information.

12. DR 33 - Dr. H.A. Hunter

"The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure"

THE CHAIRMAN said that Dr. Hunter had been invited to present his report in person but that he had been unable to do so because of his university duties at this time. The Chairman therefore presented the summary and the report, both of which appear in

APPENDIX "F"

DR. THOMSON said there was an apparent discrepancy between para. 3 of the summary and para. 4 of the report as to the number of cases treated







with  $\text{Ca}(\text{OH})_2$ .

THE CHAIRMAN observed that this study is a clinical project in which inflammatory changes may have interfered with the results.

DR. MACDONALD said he believed Dr. Hunter had used magnesium hydroxide with equally good results.

DR. GARVIN reported that "Jodoformagen" had been used by dentists for many years and that Dr. Hunter is trying to see whether some single chemical like calcium hydroxide would not do just as well as the proprietary article.

13. DR 34 - Dr. J.P. Lussier

"Studies of calcium metabolism in tooth with the aid of  $\text{Ca}^{45}$ "

This project which formerly stood in the name of Dr. A. D'Iorio and Dr. J.P. Lussier, is now being carried on entirely by Dr. Lussier.

The summary of work submitted by Dr. Lussier was read by the Chairman. The summary and a more complete report both appear in  
APPENDIX "D"

THE CHAIRMAN reported that one paper has been published on the work of this project and a second entitled "Radioactive isotopes in dental research" is now in press. A copy of the manuscript of this latter paper comprising 22 pages and 109 references is on file in the office of the Secretary of the Committee.

14. DR 35 - Dr. J. B. Macdonald

"Experimental fusospirochetal infection with mixtures of pure cultures"

DR. MACDONALD presented his own summary and report on this project, both of which appear in  
APPENDIX "J"

LUNCHEON ADJOURNMENT 12:45 p.m. to 2:00

15. DR 36 - Dr. G. Nikiforuk

"Demineralization of hard tissues as bone and teeth by organic chelating agents"

THE CHAIRMAN said that projects DR 36 to DR 39 which have still to be considered are all new projects this year. He then presented the summary and report of project DR 36. These documents appear in  
APPENDIX "C"

THE CHAIRMAN said he hoped it would be possible to have the grantee under this project present at the next meeting of the Committee.





16. DR 37 - Dr. R.E. Moyers

"Further studies in the electromyography of the head, face and neck"

THE CHAIRMAN reported that Dr. Moyers is at present in Greece and had therefore been unable to prepare a report for presentation at this time. He expected that a report on Dr. Moyers' project would be available for the Spring meeting.

17. DR 38 - Dr. K.J. Paynter

"The effect of thiouracil on the development of molar teeth of rats"

THE CHAIRMAN presented a summary of the work on this project which appears in APPENDIX "B"

18. DR 39 - Dr. A.G. Racey

"Sectioning of teeth and surrounding structures"

THE CHAIRMAN presented the summary submitted by Dr. Racey which appears in APPENDIX "E"

DR RAE thought the subject of the research should be defined.

THE CHAIRMAN said that the title given in the Appendix was the short title proposed by the grantee. In the outline of the proposed research the objective was defined as follows - "Investigation of the pathology of periodontium (human) and of teeth and surrounding structures (of dogs) deprived of normal adequate amounts of piridoxine and of normal amounts of potassium."

DR. BURTON suggested that a more suitable title for the project might be "Effects of piridoxine deficiency on teeth".

DR. RAE suggested that where the Committee is dealing with a new project it would be helpful to know what had prompted the grantee to undertake the work which he proposed to do.

THE CHAIRMAN said that an effort would be made to obtain more information on this project.

DR. BURTON suggested that a copy of Dr. Leblond's report be sent to Dr. Racey as a guide to part of the work which he is undertaking.

19. Fellowships

THE CHAIRMAN said that two fellowships had been awarded by the Committee this year and interim reports had been secured regarding the work done to date under these awards.





(i) Dr. Willa Liu Chou

In connection with the Fellowship held by Dr. Willa Liu Chou who had a grant of \$2200 for a study of migration of tooth germ during and before mixed dentition, it had not been possible to begin this work promptly owing to the absence in Europe of Dr. R.E. Moyers who was to supervise the investigation. However, Dr. Liu had submitted a brief report which appears in

APPENDIX "N"

DR. BURTON was extremely sceptical of the possibility of using a borrowed series of X-rays. He felt that the X-ray pictures used should be made under the direction of the supervisor of the project.

DR. THOMSON said that at Western Reserve University they have a long series of X-rays for use in making cranial measurements. The advantage of using existing material was that much time was saved; otherwise it would take fifteen years to obtain a complete set of pictures.

THE CHAIRMAN said that good equipment is now available at Toronto and it is believed that comparisons can be made with the Western Reserve records since Toronto has instruments similar to those used at Western Reserve and also to those at Ohio.

(ii) Dr. Gerald Shklar

THE CHAIRMAN said that Dr. Shklar had just begun work and that the Secretary had received two brief statements one from Dr. Irving Glickman and the other directly from Dr. Shklar. These two documents appear in

APPENDIX "O"

(iii) Travel Grants to Fellows

THE CHAIRMAN said that Dr. Liu had suggested that she would be applying for a travelling grant to defray her expenses in connection with the selection of X-ray materials at the universities with which she hoped to co-operate. This raised the question as to policy in regard to travel grants. In respect of travel required by grantees holding awards from the Committee, it had been established, as the policy of the Committee, that each individual request for such assistance should be dealt with on its merits and any money voted for such purposes should be taken from the Committee's travel appropriation and not be part of the research grant.

DR. THOMSON said there were two problems to be considered (i) where the grantee needs to go somewhere for information in connection with his project; (ii) where a grantee from a distant point is required to travel a long way to the university where he is to hold his fellowship.

DR. J.B. MARSHALL, Awards Officer, who was invited to join the Committee at this stage, said that applications for travel should be separated from grants-in-aid and that the same procedure applied to travel under fellowships. He pointed out that the National Research Council provides travel allowances for fellowships and he thought the same procedure should be followed in connection with dental fellowships.







He added that the fellowship announcement form is being modified and that the new form will indicate the extent of the assistance that may be granted in bringing fellows from distant points to the centres where their awards are to be held. He thought it was quite good practice for the Dental Committee to provide travel allowances under the authority of the Chairman when, in his opinion, beneficial results in connection with the grant or fellowship might be obtained by that means.

## 20. Finances

THE CHAIRMAN asked for a statement of the Committee's finances.

THE SECRETARY reported that as at 31 August 1951 the financial standing of the Committee was as follows:-

Excluding \$5,000 set aside for fellowships  
the amount awarded to the Committee by  
NRC for 1951-52 was . . . . . \$35,700.00

Of this sum, a total of . . . . . 2,846.83  
was in the possession of the grantees  
1 April 1951, and was therefore a first  
charge against their awards for 1951-52,  
leaving a balance of . . . . . 32,853.17

|                    |                                                     |
|--------------------|-----------------------------------------------------|
| Expenditures of    | \$7,952.32 (includes \$1850 on Fellowships)         |
| and Commitments of | 19,889.86 " "                                       |
| to 31 August 1951  |                                                     |
| add up to          | <u>\$27,842.18</u> (includes \$3700 on Fellowships) |
|                    | \$27,842.18                                         |

Leaving a free balance of . . . . . \$ 5,010.99

## 21. Estimates, 1952-53

It was MOVED by Dr. Charron and SECONDED by Dr. McDougall,

That the National Research Council be requested to provide in the Estimates for 1952-53 a sum in the same amount as was provided in 1951-52 for the Associate Committee on Dental Research made up as follows:

|                      |                 |
|----------------------|-----------------|
| Grants-in-aid        | \$32,500        |
| Fellowships          | 5,000           |
| Travel               | 2,500           |
| Printing proceedings | 200             |
| Contingencies        | 500             |
| Total                | <u>\$40,700</u> |

## 22. Applications for Grants, 1951-52

THE CHAIRMAN presented several new applications for grants which were





dealt with as follows:

| <u>Grant No.</u> | <u>Grantee</u>                                                                                             | <u>Short Title</u>                                                                                          | <u>Amount</u> |
|------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|---------------|
| DR 40            | Dr. L.A. Funt<br>Georgetown University<br>Washington, D.C. and<br>Dr. G.E. Sayers<br>University of Toronto | Effect of tooth eruption<br>and function on the<br>growth of the mandible<br>and facial complex on<br>cats. | \$325         |

THE CHAIRMAN stated that these two applicants are graduate students in orthodontics at Toronto and that the grant is required to provide for animals, food and denuding of skulls.

It was MOVED by Dr. Rae and SECONDED by Dr. Bagnall,  
That this application be APPROVED.

|       |                                                                            |                                                                                                                                     |       |
|-------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|-------|
| DR 41 | Dr. H. Jenkins<br>Department of<br>Orthodontics,<br>University of Toronto. | A study of the dental<br>arch form and certain<br>of its anatomical relations<br>in a series of clinically<br>excellent dentitions. | \$300 |
|-------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|-------|

THE CHAIRMAN read the outline of the proposed research which is as follows:

Thirty clinically excellent dentitions will be surveyed by means of a modified Stanton surveyor to define the individual dental arch forms, and their anatomical relations to each other, to mesio distal dental dimensions, and to the bony canine fossa points of Howes, the subspinale and supramentale points of Downs and to the mandibular notch points at the level of the occlusal plane.

It was MOVED by Dr. MacLean and SECONDED by  
Dr. Kilburn,  
That this grant be APPROVED.

|       |                      |                                                                                          |       |
|-------|----------------------|------------------------------------------------------------------------------------------|-------|
| DR 36 | Dr. Gordon Nikiforuk | Demineralization of<br>hard tissues as bone<br>and teeth by organic<br>chelating agents. | \$350 |
|-------|----------------------|------------------------------------------------------------------------------------------|-------|

THE CHAIRMAN stated that this was an additional grant under DR 36 for the purchase of equipment required in the research.

It was MOVED by Dr. Thomson and SECONDED by  
Dr. Charron,  
That this additional grant of \$350 be APPROVED.

|       |                     |          |      |
|-------|---------------------|----------|------|
| DR 13 | Dr. M. Doreen Smith | Supplies | \$75 |
|-------|---------------------|----------|------|





THE CHAIRMAN explained that Dr. Smith had found it necessary to pay dentists' assistants for the preparation of teeth which were used in her investigation and this additional grant is for that purpose.

It was MOVED by Dr. Garvin and SECONDED by  
Dr. Kilburn,  
That this additional grant be APPROVED.

DR 38 Dr. Kenneth J. Paynter

THE CHAIRMAN said that Dr. Paynter had asked permission to transfer \$800 of his grant which had been marked for salaries to supplies. The grantee was at Columbia University when he made his application and was therefore unable to be quite certain about the distribution of his expenses under the grant.

It was MOVED by Dr. Bagnall and SECONDED by  
Dr. Charron,  
That the reallocation of funds requested by  
Dr. Paynter be APPROVED.

23. Dr. Bagnall's Bibliography on Caries Research

THE SECRETARY read a letter from Dr. Allan Stralfors of the Royal Dental School, Stockholm, Sweden, stating that he was very much interested in Dr. Bagnall's Bibliography and would like to have a copy. The Secretary said that he had replied to this letter sending a copy of the booklet Trends in Caries Research and sample pages from the Bibliography and had said that he would bring Dr. Stralfors' letter to the attention of the Committee.

It was MOVED by Dr. Macdonald and SECONDED by  
Dr. Burton  
That a complimentary copy of Bibliography on  
Caries Research be sent to the Royal Dental  
School at Stockholm, Sweden. CARRIED.

THE SECRETARY reported that 153 copies of Dr. Bagnall's book had now been distributed including 33 complimentary copies and 120 sales.

There was some discussion on the desirability of securing additional copies of this very valuable publication and the Secretary reported that the plates from which it was printed were still available.

It was MOVED by Dr. Charron and SECONDED by  
Dr. Rae,  
That inquiry be made of the publishers  
regarding the cost of printing 200-300  
additional copies of Dr. Bagnall's  
Bibliography and that the Executive be  
empowered to order extra copies.

CARRIED





24. Air Travel (1951-52)

It was AGREED to include the name of Dr. A.C. Burton among those from whom air travel this year is authorized.

25. Travel to Scientific Meetings

The Committee discussed the question of providing travel funds for the use of grantees under dental committee projects so that they might be able to attend scientific meetings where subjects of interest in connection with their projects are on the program for discussion or at which the grantees or their research assistants might be enabled to present an account of the work being done under their projects.

It was MOVED by Dr. Burton and SECONDED by Dr. MacLean,

That the Associate Committee on Dental Research establish three Dental Research Special Travel grants, not to exceed \$250 each to cover travel expenses to be incurred by grantees or their research assistants in going to the annual meeting of the International Association for Dental Research at which it would be possible for them to present a report on their dental projects or to take part in discussions relevant to their researches, and

That applications for these travel grants for the current year be received up to 15 December 1951.

CARRIED.

It was further MOVED by Dr. Garvin and SECONDED by Dr. McDougall,

That the Executive be empowered to act as a selection Committee to deal with the applications for these special travel grants.

CARRIED.

26. Visitors at Next Meeting

It was suggested and AGREED that the grantees invited to attend the next meeting of the Committee include: Dr. H.A. Hunter, Dr. W.J. Linghorne, Dr. Gordon Nikiforuk, and Dr. O.J. Walker.

27. New Projects

There was a general discussion on new projects. DR. GARVIN said there was a great need for funds to train research workers.

DR. RAE pointed out that the Dental Committee is directly interested in research and that the funds provided to its grantees enable some graduate workers to obtain additional experience.





THE CHAIRMAN said that the Committee's program was in effect a combination of research and training. Three projects at the University of Toronto on the health side are supported by the Department of National Health and Welfare and he thought it was important that information should be obtained on the health factors which most largely contribute to dental disease.

DR. KILBURN said that, in his department, officials were studying the records of a group of ex service men and he would appreciate advice from the Committee as to possible uses that might be made of the data obtained in this way.

THE CHAIRMAN suggested that when the data become available he would like to have a report on this work presented to the Committee for consideration.

It was AGREED to add to the Committee's list of suggested research projects the following item:

Proposed at this meeting by Dr. A.C. Burton:  
Social aspects of dental disease.

28. Date of Next Meeting (Min. 36, 13th Mtg.)

It was AGREED that the next meeting of the Committee shall be held 3-4 March, 1952.

The meeting adjourned at 4:45 p.m.





## APPENDIX "A"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Investigations of materials for use as cements for methacrylate inlays and of methods for the use of methyl methacrylate as direct dental fillings.

Grantee: Dr. A.E.R. Westman

Projects: DR 12 and DR 25                      Amount of Grant: \$4,000

#### SUMMARY OF WORK

This report covers work done only in the first quarter of the fiscal year, since both Projects DR 12 and DR 25 were terminated as of June 30, 1951 and the remainder of the grant made available for other projects of the Committee. Mr. D.R. Christie, who had been responsible for the experimental work on various dental research projects at the Foundation since 1945, under the supervision of Mr. O.J. Schierholtz, joined the staff of the Standard Chemical Company Laboratories as of May 14, 1951. His very careful and successful efforts in connection with the various dental research projects is gratefully acknowledged.

The work which was done on the authorized projects consisted in completing some loose ends of experimental work, the writing of two papers, including the preparation of data and illustrations, and making the necessary arrangements for the termination of the fellowship.

One paper was prepared on the subject of relining acrylic dentures. The experimental work on this topic was completed in October 1950 under Project DR 21 and a final report was submitted at that time. This paper was entitled "Relining Acrylic Dentures without Distortion" and appeared in the July 1951 issue of the Journal of the Canadian Dental Association. A summary is as follows:

"It is submitted that, where thermal effects are not a factor, the distortion is caused by the added reline resin warping the original denture base primarily by polymerization shrinkage.

"                      Some degree of flexibility is required in the reline resin to overcome this distortion.

"                      Two low-temperature curing methods are suggested which by the production of flexible polymers eliminate distortion and yet assure dimensional stability".

The second paper based on the work of DR 25 and entitled "Acrylic fillings - General comments and some experimental data" was published in the August 1951 issue of the Journal of the Canadian



Dental Association. It discusses briefly the physiological effect of direct acrylic fillings on the pulp of vital teeth, the chemistry of the materials and presents some experimental data on adhesion and volumetric shrinkage. The facts would indicate that during the first hour of polymerization in the mouth, when volumetric shrinkage is greatest, real chemical adhesion exists between the filling material and the tooth dentine. If a suitable matrix material is used to compress the filling and the cavity is prepared under the driest possible conditions, the shrinkage should be localized to the free surface adjacent to the matrix and good marginal adaptation should be realized.

A.E.R. Westman

August 23, 1951.



## APPENDIX "B"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: The effect of thiouracil on the development of molar teeth of rats.

Grantee: Dr. K.J. Paynter

Project: DR 38                      Amount of Grant: \$1850.

#### SUMMARY OF WORK

This work was undertaken to determine the effects of an insufficiency of Thyroid hormone on the development of molar teeth of rats.

Thyroid insufficiency was produced by daily subcutaneous injection into the experimental animals of 6-n-propyl-2-thiouracil. Injections were started not later than twenty-four hours after birth, and maintained until sacrifice of the animal. Animals were killed when five, ten, fifteen, twenty, and twenty-five days old, and the heads prepared for study.

Evidence of lack of thyroid hormone has been determined from the daily weight records of the animals, and from study of sections of their pituitary and thyroid glands.

Grenz ray pictures of one half the mandible show marked differences in mandibular bone density, as well as variation of the positional relationship of the tooth crypts within the mandible.

The rest of the head of each animal has been imbedded in paraffin preparatory to sectioning for microscopic study.

To date this work has been done at the Department of Anatomy of Columbia University, New York, and has been financed by that institution.

K.J. Paynter

28 August, 1951.





## APPENDIX "C"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Demineralization of hard tissues as bone and teeth by organic chelating agents.

Grantee: Dr. G. Nikiforuk

Project: DR 36

Amount of Grant: \$2400

#### SUMMARY OF WORK

Preliminary investigations have shown that demineralization of bone tissue can be accomplished at varying rates, by the use of saturated solutions of Versene (ethylene-diamine tetra acetate), at pH's ranging from 5.0 to 10.3. The solubility ranges from 11 g/100 ml. water for the disodium salt (pH 5.0) to 103 g/100 ml. water for the tetrasodium salt (pH 10.3). The time required is about the same as that needed for a comparable specimen in the formic-citric acid method of Morse, and slightly longer than that required for 5% HNO<sub>3</sub>. Preservation of structural detail and of the staining properties of the tissues seems excellent. This was particularly evident in the elements of the pulp and bone marrow. Further experiments to determine the optimal pH and concentration of the chelating compounds for the preservation of tissue elements are now being conducted.

The staining properties of the tissues demineralized by Versene appear to be strikingly different from similar specimens decalcified by acid solutions. This difference is probably a function of pH. Depending upon the isoelectric point of the protein, pH may alter the electric charge on the molecule, and consequently alter its affinity for dyes, (eosin-hematoxylin used in this study). The histological phase of this project is being investigated.

Gordon Nikiforuk

16 August, 1951.





## APPENDIX "C"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Demineralization of hard tissues as bone and teeth by organic chelating agents.

Grantee: Dr. G. Nikiforuk

Project: DR 36

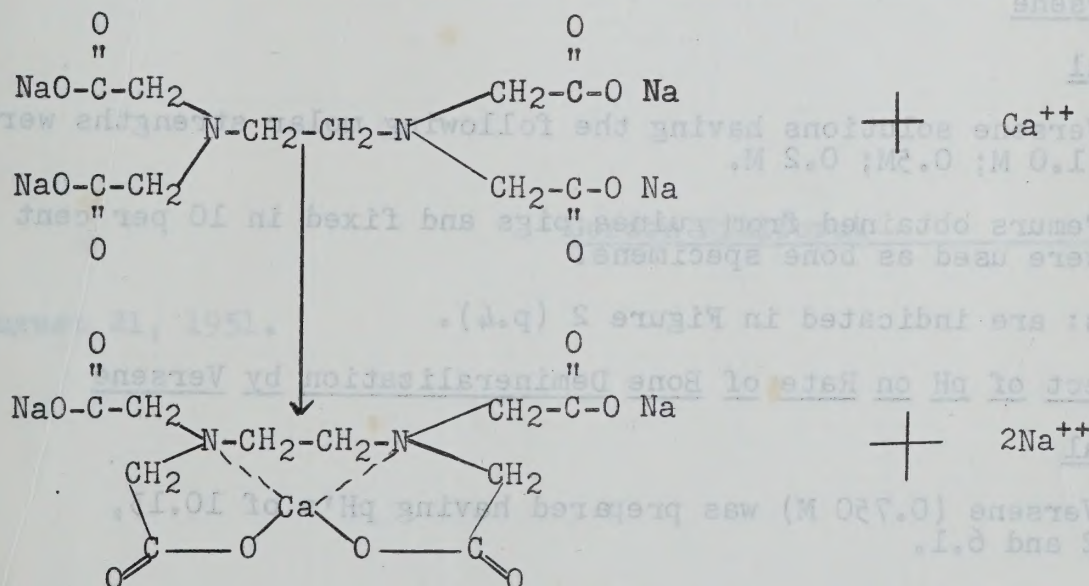
Amount of Grant: \$2400

#### I. Introduction

Previous histological and histochemical studies of bone and other hard tissues have been limited to tissue sections prepared by acid decalcification. The object of this research project is to investigate the ability of some organic chelating agents to make suitable demineralizing media for hard tissues.

#### II. Material

The chelating agent used in this study is the sodium salt of ethylene diamine tetra acetic acid (trade name -Versene). The compound has the property of forming soluble nonionic chelates with a large number of metallic ions. The reaction with calcium is thought to proceed as follows:



Versene + Metal Ions → Metal Versene Complex + Sodium Ions



### III. Experimental Findings

#### A. Effect of pH on Complexing Power of Versene

The complexing power of Versene, towards metallic ions, is most efficient in alkaline solutions. As the pH is reduced the stability of the complex decreases. The relation of pH to the complexing power of Versene towards calcium is shown in Figure 1(p.4).

#### B. Demineralization of Hard Tissues by Versene: Preliminary Experiment

##### 1. Materials Used

A 0.78 Molar solution of Versene was prepared by neutralizing a 34 per cent solution of Versene (sodium salt) with HCl. A bone specimen (femur from the guinea pig) fixed in 10 per cent formalin was used.

##### 2. Result

The bone specimen was demineralized by the above solution in 90 hours. The bone specimen was considered demineralized when it became suitable for histologic sectioning.

A similar specimen dipped in 5 per cent HNO<sub>3</sub> decalcified in 50 hours.

#### C. Effect of Concentration on Rate of Bone Demineralization by Versene

##### 1. Material

Versene solutions having the following molar strengths were prepared: 1.0 M; 0.5M; 0.2 M.

Femurs obtained from guinea pigs and fixed in 10 per cent formalin were used as bone specimens.

2. Results: are indicated in Figure 2 (p.4).

#### D. Effect of pH on Rate of Bone Demineralization by Versene

##### 1. Material

Versene (0.750 M) was prepared having pH's of 10.13, 8.81, 7.32 and 6.1.

##### 2. Result

The results are similar to the curve in Fig. 1.

Above pH 7.5, no detectable difference in the speed of demineralization was observed; pH's below 7.3 gave slower rates of demineralization of bone. These results parallel the Calcium complexing power of Versene at various pH's.(see Fig. 1).



"C-3"

E. Histological Findings

Only a few sections are available. These suggest the following:

- a. Preservation of staining properties is excellent, particularly in the bone marrow.
- b. The specimens demineralized by Versene possess different staining properties from similar sections demineralized by 5 per cent  $\text{HNO}_3$ .
- c. Tissue demineralized by Versene is "harder". This may be due to removal of less organic material from bone than occurs when a specimen is decalcified by acid.

Comments

The histological phase of the study awaits further investigation.

Means of increasing rate of demineralization will be attempted.

Effect of temperature has not been investigated.

Teeth have not been used as yet.

Gordon Nikiforuk

August 21, 1951.





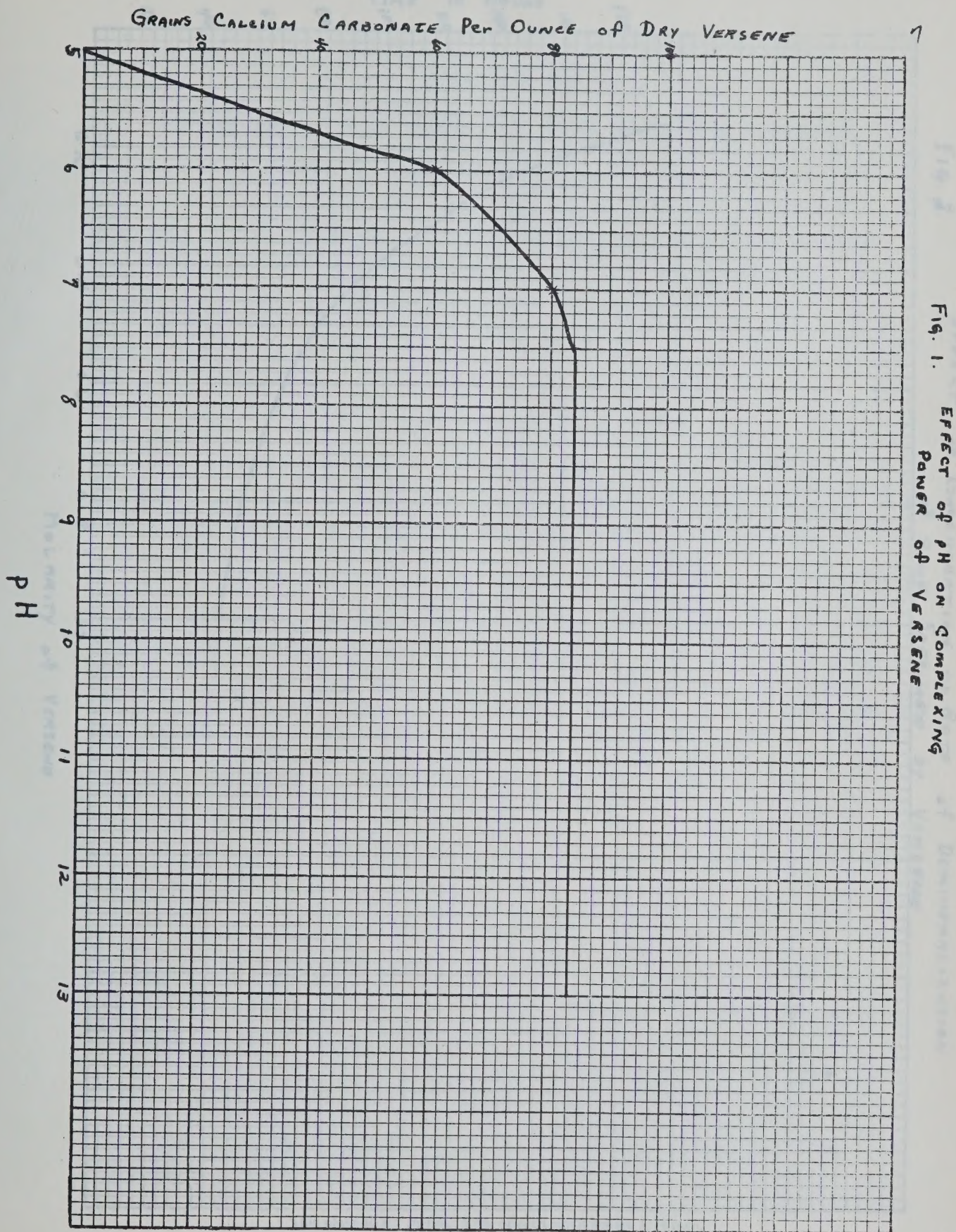


Fig. 1. EFFECT OF PH ON COMPLEXING POWER OF VERSENE

This plate 10 x 7 inches. Edge of each small square 1/10 inch.

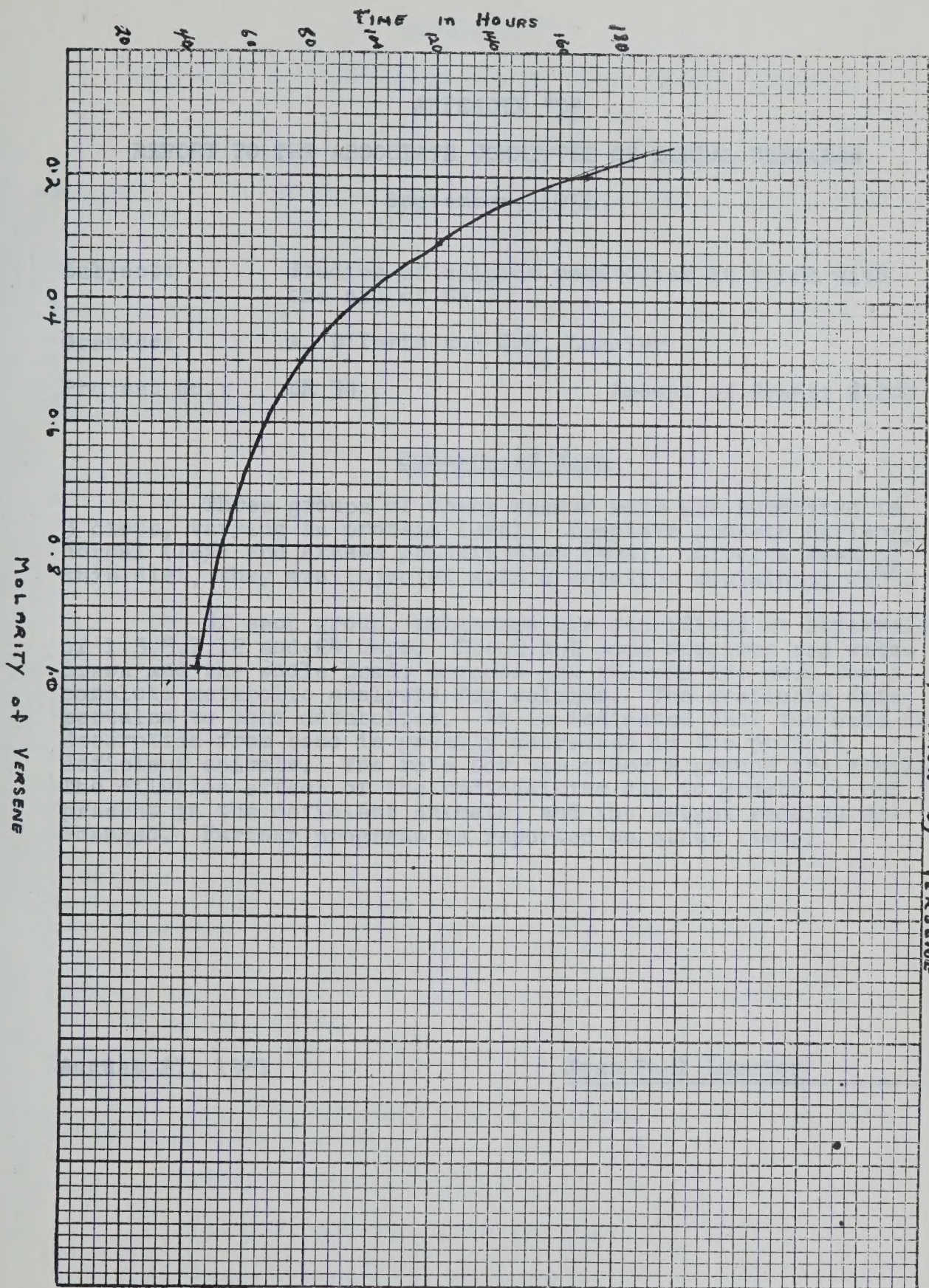
C.C.C.  
T.







FIG. 2. EFFECT OF CONCENTRATION ON RATE OF DEMINERALIZATION OF BONE SPECIMEN BY VERRSENE



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.

T.







APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

APPENDIX "D"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Subject: Studies of calcium metabolism in tooth with the aid of  $Ca^{45}$  September, 1951

Subject: Studies of calcium metabolism in tooth with the aid of  $Ca^{45}$

Grantee: A. D'Iorio and J.P. Lussier

Project No.: DR 34 Amount of Grant: \$1200

Summary of Work

Three groups of newly weaned rats are submitted to 3 diets, varying in protein level. Complete protein deficiency, normal (18%) and excess protein diet (50%). After a week of this diet, they are injected with a solution containing  $Ca^{45}$ .

Of each group, subgroups are sacrificed at intervals of 1,3,5,7,12 and 24 days. Blood and bone specimen are withdrawn and the whole head, carcass and excreta are used for a complete metabolic analysis for calcium. The available data pertains to bone metabolism. It is indicated that the rate of desorption from bone is greatly decreased in the protein deficient animals. The rate for the other 2 groups are similar. The dilution effect of the radiocalcium due to growth is not present in the deficient animals, and the weight remains unchanged. Further progress is reported on other items.

A complete survey of the calcium metabolism was undertaken in animals receiving 1) a normal diet, 2) a protein deficient diet, 3) a diet containing excess protein. There is no data in the literature of these types of diet variations (3). A copy of a comprehensive review of the work done with radioactive isotopes in dental research is found in the present report. This review will soon be published in England by the British Dental Annual.

August 27, 1951

Jean Paul Lussier

Newly weaned male rats (30-40gms) are divided into three main dietary groups.

Group A normal diet.

Group B protein deficient diet.

Group C excess protein diet.





## APPENDIX "D"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1951

Subject: Studies of calcium metabolism in tooth with the aid of  $\text{Ca}^{45}$

Grantee: A. D'Iorio and J.P. Lussier

Project No.: DR 34

Amount of Grant: \$1200

#### Introduction -

A report was already published (1) in connection with this project, and it was shown that repeated injections of radioactive calcium induce a regular deposition of the radioactive element in the calcified tissues. About 0.25% of each injected dose is recovered in the highly calcified structures of the teeth. While 1% is found in the less calcified ones. However, as already pointed out by Hodge (2), it is still indefinite as to what proportion of the calcium is 1) stored in the calcified areas, 2) incorporated in the calcifying or growing largers or 3) simply exchanged with the biological fluids by mean of adsorption. The rate of exchange is conditioned by the crystalline surface in contact with the fluids, (when all other factors as temperature, concentration and specific surface are equal). It is therefore assumed that the difference in the calcium fractions between a growing group and a non growing group, may be due to growth when values for both groups are reported to weight unity or to surface unity. A complete survey of the calcium metabolism was undertaken in animals receiving 1) a normal diet, 2) a protein deficient diet, 3) a diet containing excess protein. There is no data in the literature of these types of diet variations (3). A copy of a comprehensive review of the work done with radioactive isotopes in dental research is found in the present report. This review will soon be published in England by the British Dental Annual.

#### Experiments -

Newly weaned male rats (30-40gms) are divided into three main dietary groups.

Group N normal diet,

Group D protein deficient diet,

Group S excess protein diet.



These animals receive the respective diet for a week prior to the injection of radioactive calcium. For time factor studies, each group is subdivided into subgroups of 4 or 5 animals at the beginning of the experiments. A subgroup distribution chart is shown in Table I. Body weight variations in relation to diet appear in Table II.

A  $\text{Ca}^{45}(\text{NO}_3)_2$  solution supplied by the Eldorado Mining Corporation with a radioactivity of 48 mc/gm is neutralized and diluted to 50 ml. This solution has an activity of 32,582,500 C.P.M. Each animal is injected intraperitoneally with half a ml. of the solution. It receives a radioactive dose of 325,825 C.P.M. The subgroups are sacrificed at intervals of 1, 3, 5, 7, 12 and 24 days. The subgroup D6 did not survive for 24 days and is excluded. The animals are sacrificed with ether. A blood sample is withdrawn by heart puncture, tibiae and humeri are excised. The head is cut away.

#### Radioactivity measurements -

The following are studied for radioactivity

- a) blood,
- b) long bones (tibia and humerus),
- c) teeth (head),
- d) carcass (remaining parts of the body),
- e) excreta (urine and feces)

#### Calculation -

All radioactivity measurements last 20 minutes. The calculation is made according to the following formula:

$$\frac{\text{C.P.M.} - \text{C.P.M. (background)}}{W} \times K \times K_1 = \text{C.P.M./mg at 0 thickness}$$

C.P.M. = Counts per min. of the sample spread on the cupule,

W = weight of sample on the cupule in mgs.

K = correction for absorbed radiations due to the thickness of the sample. It is calculated by this formula:

$$K = \frac{W \times 0.126}{\text{surface of the cupule}} + 1$$

$K_1$  = correction for the rate of decay.



## METHODS AND RESULTS

### a) Blood -

From each subgroup, blood samples are pooled, and trichloroacetic acid is added. The mixture is boiled for three hours, filtered and adjusted to volume (10 ml.) One ml. is evaporated on aluminium cupule for radioactivity determinations. The removal of the radioactive element from the blood is very rapid. Therefore the first readings after 24 and 72 hours were inconclusive. For exact values, blood samples should be removed after the injection at every two hours for a total observations period not exceeding 36 hours. The peak of radioactivity in the blood is reached within two days following the injection of radiophosphorus(4). Calcium withdrawal from the blood may be more rapid, as it is present in serum only.

### b) Long bones -

Tibiae and humeri are dried in a vacuum oven at 75°-80°C for 24 hours. With a diamond disc these bones are cut longitudinally for the removal of the bone marrow and then, transversally at the epiphyso-metaphyses and metaphyso-diaphyseal junctions. The isolated epiphyses, metaphyses and diaphyses are prepared for radioactive determination of the effect of the injections on the calcium metabolism. Each portions is weighed, dissolved in conc. HCl, filtered to removed protein, and diluted to 25 ml. 20 ml. aliquots are neutralized by NH<sub>4</sub>OH. Saturated ammonium oxalate is added to initiate precipitation of calcium. After centrifugation, the precipitate is washed, dried under vacuum at 70° - 80° C, mashed with toluene in a mortar and spread on a previously weighed aluminium cupule. The cupule is dried under an infra-red lamp to complete evaporation. After cooling at room temperature, the cupule is weighed again, and the radioactivity determined. Values in C.P.M. (mg of Ca oxalate at zero) thickness are reported in Table III. Calcium determinations of these samples are not complete.

### c) Teeth -

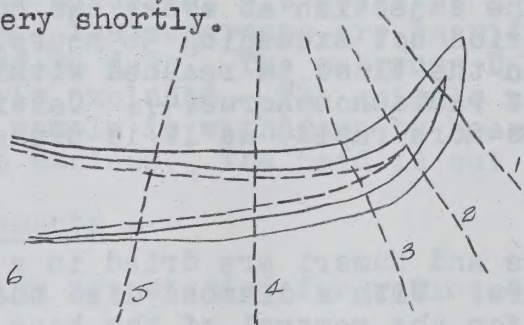
A main difficulty is the adequate separation of the dental tissues by the Manly and Hodge's technique (5) for enamel and dentin separation. Some criticisms of this method have been reported even for human teeth. Berggren (6) recommends Pickerill's technique (7), but it is not applicable to very small teeth. Gilda (8), recently introduces slight modifications to the Manly's technique in order to make it usable for rodent teeth. However, he did not pay any attention to the incisors. Density values reported in the dental literature are very scarce (9). A modified method must be used for the complete separation of enamel and dentin either radicular or coronary, before any attempts be made for radioactivity measurements.



The following scheme is proposed for the separation of enamel and dentin by mean of their respective densities:

A.- Molars, to follow Gilda's technique (8) and improve it if necessary.

B.- Incisors, to determine in the different cuts as shown in the following diagram the percentage of the weight which is sedimentating at different density intervals. This application of the Manly's technique should be undertaken, unless a future report would mention it very shortly.



#### d) Carcass -

The carcass are ashed in a muffle furnace. Calcium determinations and radioactivity measurements are to be made.

#### e) Excreta -

Urine and feces are collected for each subgroup. The whole mass is first ashed in a muffle furnace. Then, it undergoes a prolonged digestion in a  $\text{HNO}_3\text{-HCl}$  mixture, afterwards, it is filtered. The filtrate is neutralized, mixed with a saturated ammonium oxalate solution. The precipitate is filtered, washed in the  $\text{NH}_4\text{OH}$  5%, dissolved in  $\text{HCl}$  20%, filtered again to remove colloidal organic material. The calcium is again precipitated by the same procedure. This time the precipitate is ashed in muffle furnace to remove all traces of organic material. The calcium, now in the form of carbonate, is very soluble in  $\text{HCl}$ . A final precipitation with oxalate brings a crop of pure calcium oxalate crystals which can be determined either for titration or radioactivity measurement. This final step is still to be performed.

#### In vitro experiments -

The influence of various substances on the rate of calcium penetration into the human tooth has been studied. Glucose and urea seemed the most interesting because they dealt with Berggren's (5) and Wainwright's (10) studies. Preliminary study of the work did not bring conclusive support to our hypothesis as reported previously.



Influence of already adsorbed fluorine on the rate of penetration of calcium in dental enamel was also undertaken. Dr. Rae and Dr. Hunter's suggestion to use fluorinated teeth has been appreciated. We have just recently received fluorinated teeth from Stratford. We are greatly indebted to Dr. Brown for his kind assistance in finding this precious material. These will be used in vivo experiment.

#### Autoradiographs -

Though there is some progress in this field, the time consuming technique, has not given satisfactory results. Gross and histological autoradiography of tibiae and humeri, whole head, rat teeth and human teeth must be made to confirm in vitro results. Clear cut results would then be publishable.

#### Comments -

Although complete results are lacking, data concerning radioactivity in the different parts of long bone, indicate a rapid desorption from the epiphyses. Graphs I and II, show a slow desorption phase in the deficient subgroup, with no appreciable differences for the N and S subgroups. This also applies to the tibia and humerus. Though the desorption rate seems to be slower in the humerus than in the tibia. This fact is more obvious in Graph II when the D curve is almost straight line. These differences may be due to a dilution factor of  $\text{Ca}^{45}$  in the total calcium. N and S groups are growing normally, while D groups show no growth. This lack of increment of bony surface, presents further dilution of Calcium<sup>45</sup>, and the only effect observed seems to be due to desorption alone. N and S groups would show a desorption effect plus a dilution effect related to growth metaphyses radioactive data show a desorption phase at the beginning followed by an apparent readsorption more obvious at the fifth or seventh day after the injection. As pointed out by Leblond et al. (11) with the aid of autoradiographs, the tide of radioactive calcium is directed from the epiphyses to the diaphyses. The metaphyses being on the way, may therefore be invaded secondarily while transformation of spongy bone to compact bone occurs. The  $\text{Ca}^{45}$  concentration does not seem to vary appreciably about the diaphyses. The desorption rate is probably parallel to the adsorption one, and minor changes that may occur during the investigating period are not noticeable since the values do not show significant variations.

Conclusions, however, will have a better support when data on specific activity are available. Values reported to weight or surface unity will also help determining more precisely the boundaries between both phenomena.



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"D-7"

TABLE I

GROUP DISTRIBUTION CHART

| % Protein in diet | Survival days following injection of $\text{Ca}^{45}$ |       |       |       |       |       |
|-------------------|-------------------------------------------------------|-------|-------|-------|-------|-------|
|                   | 1                                                     | 3     | 5     | 7     | 12    | 24    |
| (Normal) 18%      | $N_1$                                                 | $N_2$ | $N_3$ | $N_4$ | $N_5$ | $N_6$ |
| (Deficient) 0%    | $D_1$                                                 | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ |
| (Excess) 50%      | $S_1$                                                 | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ |





TABLE II

| Group          |          | N         |          |         |                |          | D         |          |         |                |          | S         |          |      |  |  |
|----------------|----------|-----------|----------|---------|----------------|----------|-----------|----------|---------|----------------|----------|-----------|----------|------|--|--|
| Subgroup       | Animal # | Weight I. | Final W. | Variat. | Subgroup       | Animal # | Weight I. | Final W. | Variat. | Subgroup       | Animal # | Weight I. | Final W. | Var. |  |  |
| N <sub>1</sub> | a        | 59        | 67       | +8      | D <sub>1</sub> | a        | 35        | 35       | -1      | S <sub>1</sub> | a        | 57        | 65       | +8   |  |  |
|                | b        | 73        | 68       | -5      |                | b        | 35        | 35       | 0       |                | b        | 71        | 70       | -1   |  |  |
|                | c        | 62        | 58       | -4      |                | c        | 39        | 38       | -1      |                | c        | 58        | 50       | -8   |  |  |
|                | d        | 63        | 60       | -3      |                | d        | 43        | 42       | -1      |                | d        | 57        | 54       | -3   |  |  |
|                | e        | 70        | 65       | -5      |                | e        | 34        | 34       | 0       |                | e        | 58        | 53       | -5   |  |  |
| N <sub>2</sub> | a        | -         | -        | -       | D <sub>2</sub> | a        | 38        | 37       | -1      | S <sub>2</sub> | a        | 64        | 65       | +1   |  |  |
|                | b        | 54        | 59       | +5      |                | b        | 46        | 45       | -1      |                | b        | 52        | 52       | 0    |  |  |
|                | c        | 63        | 69       | +6      |                | c        | 36        | 35       | -1      |                | c        | 76        | 85       | +9   |  |  |
|                | d        | 69        | 78       | +9      |                | d        | 37        | 37       | 0       |                | d        | 68        | 73       | +5   |  |  |
|                | e        | 77        | 84       | +7      |                | e        | 73        | 70       | -3      |                | e        | 55        | 58       | +3   |  |  |
| N <sub>3</sub> | a        | -         | -        | -       | D <sub>3</sub> | a        | 39        | 40       | +1      | S <sub>3</sub> | a        | 66        | 75       | +11  |  |  |
|                | b        | 73        | 90       | +17     |                | b        | 42        | 42       | 0       |                | b        | 67        | 85       | +18  |  |  |
|                | c        | 76        | 90       | +14     |                | c        | 44        | 45       | +1      |                | c        | 66        | 77       | +11  |  |  |
|                | d        | 64        | 75       | +11     |                | d        | 44        | 43       | -1      |                | d        | 62        | 70       | +8   |  |  |
|                | e        | 49        | 60       | +11     |                | e        | 34        | 40       | +1      |                | e        | 60        | 64       | +4   |  |  |

"D-8"





TABLE II (continued)

N

D

S

Group

| Subgroup       | Animal # | Weight I. | Final W. | Variat. | Subgroup       | Animal # | Weight I. | Final W. | Variat. | Subgroup       | Animal # | Weight I. | Final W. | Var. |
|----------------|----------|-----------|----------|---------|----------------|----------|-----------|----------|---------|----------------|----------|-----------|----------|------|
| N <sub>4</sub> | a        | 53        | 40       | +13     | D <sub>4</sub> | a        | 44        | 35       | -9      | S <sub>4</sub> | a        | 59        | 63       | +4   |
|                | b        | 59        | 50       | +9      |                | b        | 42        | 32       | -10     |                | b        | 45        | 45       | 0    |
|                | c        | 60        | 55       | +5      |                | c        | 38        | 30       | -8      |                | c        | 48        | 53       | +5   |
|                | d        | 45        | 35       | +10     |                | d        | 36        | 28       | -8      |                | d        | 50        | 64       | +14  |
|                | e        | 60        | 45       | +15     |                | e        | 43        | 35       | -8      |                | e        | 48        | 43       | -5   |
| N <sub>5</sub> | a        | -         | -        | -       | D <sub>5</sub> | a        | 42        | 38       | -4      | S <sub>5</sub> | a        | 60        | 90       | +30  |
|                | b        | 56        | 80       | +24     |                | b        | 43        | 40       | -3      |                | b        | 68        | 108      | +40  |
|                | c        | 65        | 92       | +27     |                | c        | 40        | 40       | 0       |                | c        | 60        | 100      | +40  |
|                | d        | 63        | 98       | +35     |                | d        | 33        | 30       | -3      |                | d        | 58        | 75       | +17  |
|                | e        | 70        | 94       | +24     |                | e        | 35        | 34       | -1      |                | e        | 49        | 78       | +29  |
| N <sub>6</sub> | a        | 66        | 97       | +31     | D <sub>6</sub> |          |           |          |         | S <sub>6</sub> | a        | -         | -        | -    |
|                | b        | 55        | 109      | +44     |                |          |           |          |         |                | b        | 59        | 115      | +56  |
|                | c        | 53        | 101      | +48     |                |          |           |          |         |                | c        | 52        | 115      | +67  |
|                | d        | 67        | 128      | +41     |                |          |           |          |         |                | d        | 48        | 100      | +52  |
|                | e        | -         | -        | -       |                |          |           |          |         |                | e        | -         | -        | -    |

"D-9"





"D-10"

TABLE III

RADIOACTIVITY IN LONG BONE (C.P.M./mg)

- Tibia -

| Group      | Epiphyses |     |     | Metaphyses |     |     | Diaphyses |     |     |
|------------|-----------|-----|-----|------------|-----|-----|-----------|-----|-----|
|            | N         | D   | S   | N          | D   | S   | N         | D   | S   |
| Subgroup 1 | 673       | 862 | 587 | 306        | 523 | 255 | 110       | 147 | 98  |
| 2          | 312       | 579 | 460 | 226        | 376 | 279 | 109       | 156 | 85  |
| 3          | 294       | 496 | 326 | 266        | 465 | 255 | 125       | 154 | 106 |
| 4          | 236       | 398 | 261 | 299        | 449 | 326 | 122       | 155 | 142 |
| 5          | 130       | 384 | 139 | 138        | 392 | 165 | 114       | 141 | 104 |
| 6          | 97        | -   | 92  | 247        | -   | 120 | 117       | -   | 118 |

- Humerus -

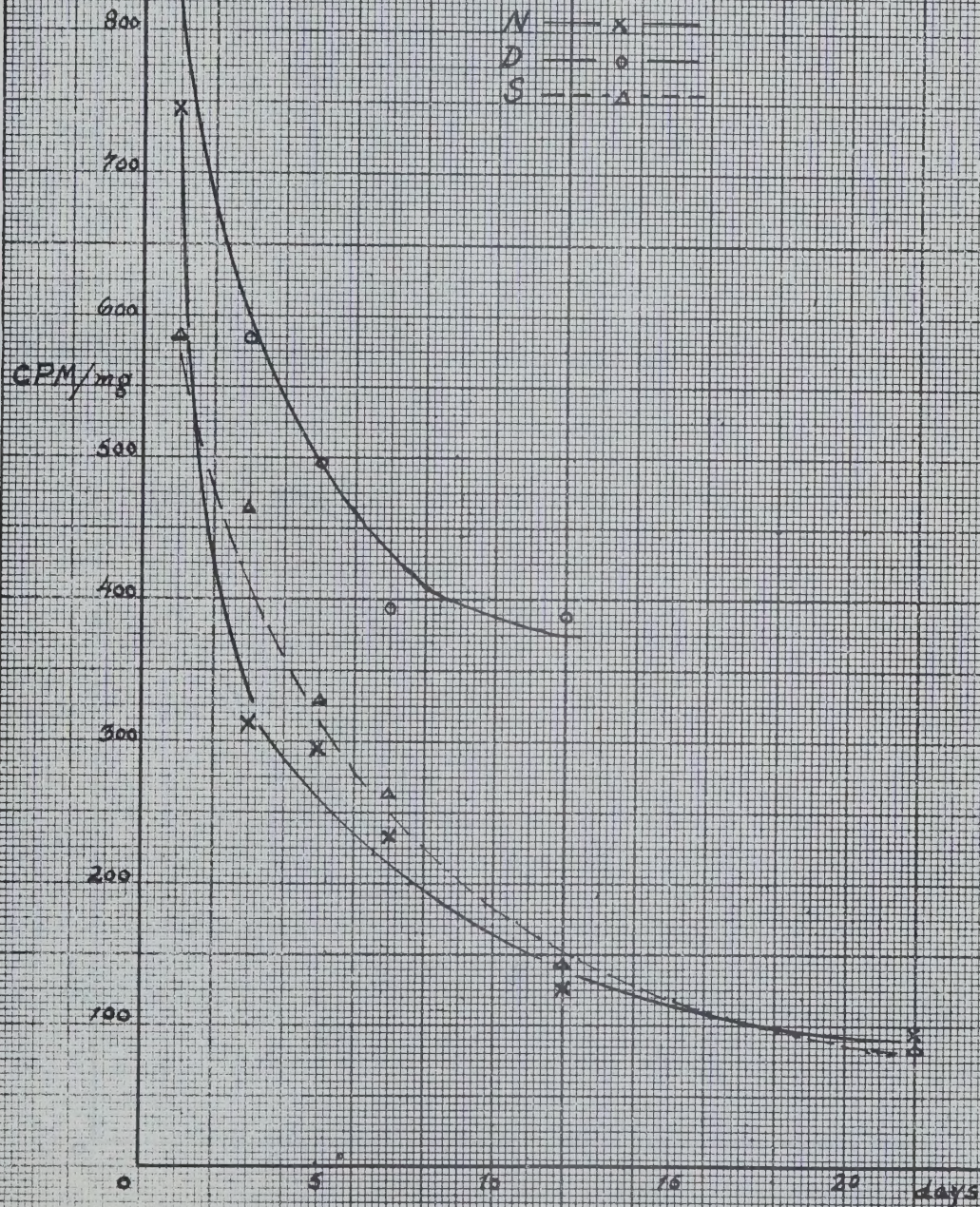
| Group      | Epiphyses |     |     | Metaphyses |     |     | Diaphyses |     |     |
|------------|-----------|-----|-----|------------|-----|-----|-----------|-----|-----|
|            | N         | D   | S   | N          | D   | S   | N         | D   | S   |
| Subgroup 1 | 603       | 595 | 441 | 225        | 334 | 163 | 114       | 157 | 82  |
| 2          | 342       | 515 | 419 | 219        | 501 | 225 | 118       | 149 | 110 |
| 3          | 259       | 453 | 307 | 215        | 316 | 204 | 131       | 134 | 113 |
| 4          | 289       | 457 | 245 | 246        | 385 | 271 | 160       | 153 | 128 |
| 5          | 123       | 369 | 126 | 132        | 329 | 106 | 95        | 144 | 107 |
| 6          | 82        | -   | 89  | 120        | -   | 106 | 83        | -   | 95  |





GRAPH I

TIBIA EPIPHYSIS



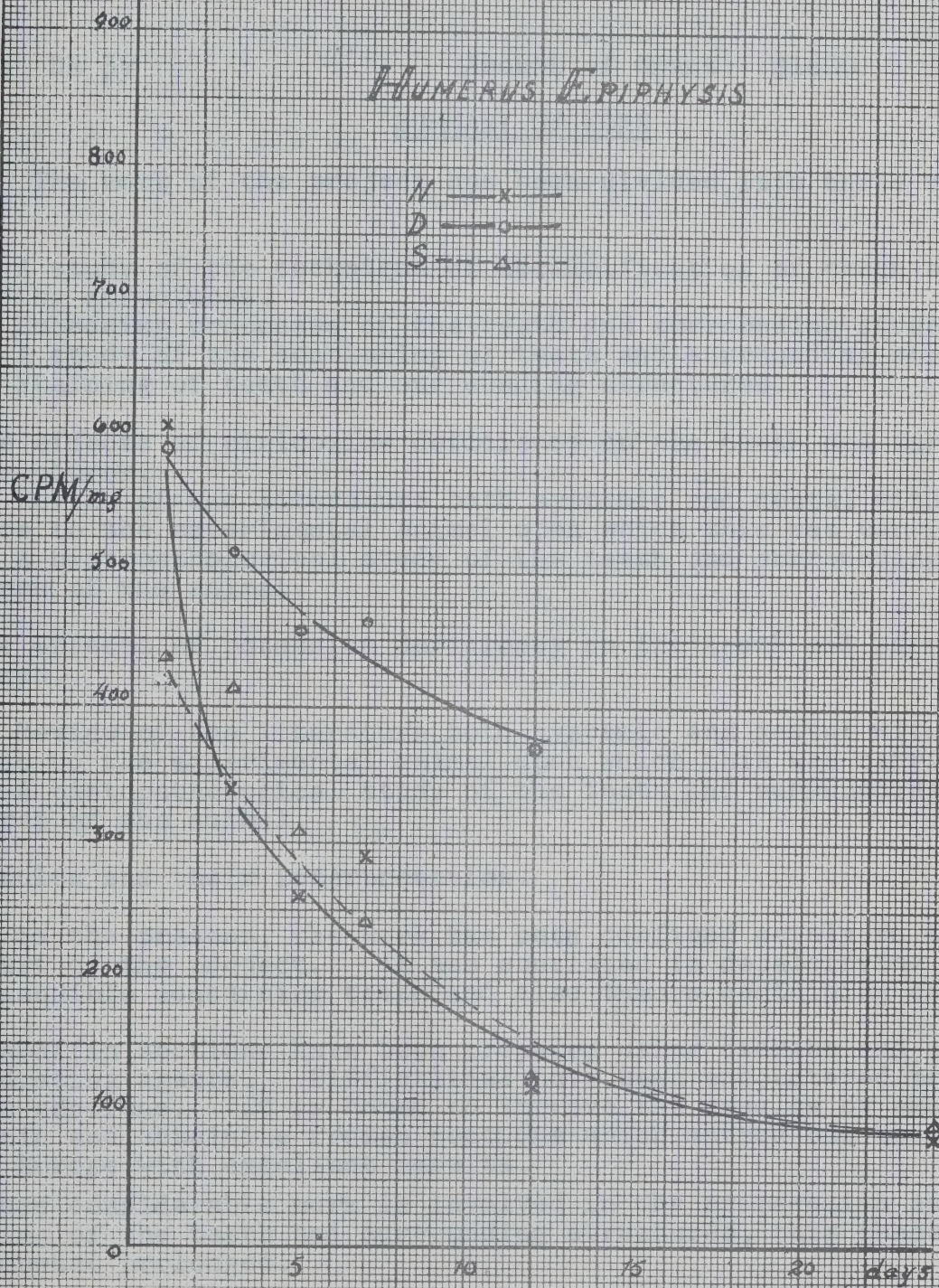






GRAPH II.

HUMERUS EPIPHYSIS









## APPENDIX "E"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Sectioning of teeth and surrounding structures

Grantee: Dr. A.G. Racey

Project: DR 39

Amount of Grant: \$1600

#### SUMMARY OF WORK

The work of the project has been divided into three main parts:

- 1) a) A study of the technique of decalcification of teeth and surrounding structures.  
b) Embedding processes of the teeth and surrounding structures  
c) A study of the sectioning of teeth and surrounding structures.  
d) Staining of teeth and surrounding structures.
- II) The effects of piridoxine deficiency on the teeth and surrounding structures.  
a) Review of the literature both systemic and dental with reference to the experimental animal upon which this was carried out.  
b) A histopathological study of the teeth and surrounding structures of the animal.
- III) The effects of potassium deficiency on the teeth and surrounding structures.  
a) Review of the literature both systemic and dental with reference to the experimental animal upon which this was carried out.  
b) A histopathological study of the teeth and surrounding structures of the animal.
- IV) Results and their interpretation.

Up to the present, the work has been confined mainly to the first part of the project. It has consisted in decalcifying sections of the mandible containing teeth and also tumors containing calcified areas.

Paraffin sections have been prepared using dioxane and alcohol as dehydrating agents. Difficulties have been encountered preparing paraffin sections. It is hoped to improve the results by embedding in gelatin and obtaining frozen sections which method has been used in Europe with excellent results.

Since considerable work is involved in the preparation and sectioning of the teeth and surrounding structures, this phase has been somewhat prolonged.

The material for the above has been obtained from the dental clinic of the Montreal General Hospital; and, the Department of Experimental Surgery of McGill University.

A.G. Racey





APPENDIX "F"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: The investigation of the mechanism concerned in the deposition of lime salts formed in bridging over a pulp exposure.

Grantee: Dr. H. A. Hunter

Project: DR 33

Amount of Grant: \$1600

SUMMARY OF WORK

To date seventy tooth pulps of dog's have been treated with seven medicaments placed directly over freshly prepared pulp exposures. Of these, twenty have been examined histologically.

The test materials are  $\text{Ca}(\text{OH})_2$ ,  $\text{Mg}(\text{OH})_2$ , "Jodoformagen", calcium oleate, cholesterol, Zinc oxide - eugenol cement and "Denco" temporary cement.

All twenty teeth examined showed inflammatory or degenerative changes in the pulp and four showed calcific bridging of the exposure. Results so far are not indicative of any trend. Two of these four cases are in teeth treated with  $\text{Ca}(\text{OH})_2$ , one with "Jodoformagen", and one with zinc oxide -- eugenol cement.

It is felt that the general reactions of the pulp, i.e. suppuration and necrosis are interfering with the true effects of the test medicaments. A modification in operative technique is proposed to avoid this feature.

H. A. Hunter

31 August, 1951.





## APPENDIX "F"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: The investigation of the mechanism concerned in the de-position of lime salts formed in bridging over a pulp exposure.

Grantee: Dr. H. A. Hunter

Project: DR 33 Amount of Grant: \$1600

#### REPORT

Following up the programme laid out in the previous annual report, seventy dog's teeth have been treated according to the schedule below. Of these, histological sections are on hand for twenty and the balance are in varying degrees of preparation.

The number of teeth examined is not as yet enough to allow anything more than a report on specific cases, though it is hoped to have numerically enough at a later date to analyse the results.

None of the sections showed normal pulps under the treatment fillings. The conditions present in the pulp included completely necrotic, advanced reticular atrophy, suppurating pulpitis, chronic pulpitis and hyperaemia. These conditions may be due in part to the prearranged plan of using a non-sterile technique in exposing the pulps of the teeth.

The results in these twenty teeth are not indicative of any trend. There are four cases showing calcific bridging of the pulp exposure; two  $\text{Ca}(\text{OH})_2$  cases and one zinc oxide -eugenol case of nine weeks duration and one "Jodoformagen" case of 5 weeks duration. All the remaining sixteen cases showed no repair of the exposure, though this was explainable in some cases by the presence of suppuration or necrosis of the pulp tissue, whose vitality is considered to be a prerequisite to healing.

None of the five cases using calcium oleate over periods of two, five and nine weeks showed any calcific deposits over the exposure. As yet none of the teeth treated with cholesterol are available for study. These were included in the study of test materials because of the common association with pathological calcific processes such as gallstones, tuberculous lesions and atheromatous plaques.

Of the two teeth examined that were treated with  $\text{Mg}(\text{OH})_2$  one was damaged by drilling through too far, resulting in a periodontal abscess and the other showed a necrotic pulp, so neither could show any repair.



"F-2"

A comparison of the  $\text{Ca}(\text{OH})_2$  and  $\text{Mg}(\text{OH})_2$  groups of teeth is designed to show the influence of pH alone which is respectively 12 and 10.3. Both of these are sufficient to precipitate the protein adjacent to it in the wound and this protein barrier may protect the rest of the tissues from precipitation. The effect of this would be to produce a pH gradient across the precipitate and to sustain an elevated pH at the periphery of the living tissue, thus lowering the solubility of calcium salts.

From an examination of the above cases it is felt that a modification of the technique of preparation and application of the test medicaments will be necessary to eliminate the disruptive effects of infection and inflammation on the pulp, otherwise the test materials may not have a chance to exert their influences on the reaction of the pulp tissues to the original injury.

H.A. Hunter

Date: 31 August 1951.



## REPORT TO THE ASSOCIATE CHIEF OF DENTAL RESEARCH "F-3"

SCHEDULE 1951

Subject: Chemical mechanisms in dental caries

|                         | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9weeks |
|-------------------------|---|---|---|---|---|---|---|---|--------|
| Calcium hydroxide J. H. | 2 | 2 | 3 |   | 4 |   |   |   | 2      |
| Magnesium hydroxide     | 2 | 2 | 2 | 2 | 2 | 2 | 2 |   |        |

SUMMARY OF WORK

|                               |   |   |   |   |   |  |  |  |   |
|-------------------------------|---|---|---|---|---|--|--|--|---|
| "Jodoformagen"                | 2 |   | 2 | 2 | 2 |  |  |  | 2 |
| Calcium oleate                | 2 | 4 | 2 | 2 | 3 |  |  |  | 2 |
| Zinc oxide-<br>Eugenol cement |   |   | 2 | 2 | 2 |  |  |  | 2 |

The research on the growth inhibitor for lactic bacillus acidophilus found in bromoform treated enamel has been continued. The paper "Ammonia Production in Saliva" by Bellantyne, Clegg, Hae and Lawford, referred to in our last report, has been published in J. Dent. Res. 30, p. 365, 1951. Reports of this paper are not yet available. A second paper, also referred to in our last report has been revised and is being submitted to the Dental Research for 2 1/2 2 2 2.

The research on the growth inhibitor for lactic bacillus acidophilus found in bromoform treated enamel has been continued. The paper "Ammonia Production in Saliva" by Bellantyne, Clegg, Hae and Lawford, referred to in our last report, has been published in J. Dent. Res. 30, p. 365, 1951. Reports of this paper are not yet available. A second paper, also referred to in our last report has been revised and is being submitted to the Dental Research for 2 1/2 2 2 2.

Cholesterol tooth enamel, the fraction 2 of the dried acetone residues which was insoluble in chloroform and the precipitate obtained by treating the above fraction with sodium hydroxide, were prepared to Eli Lilly for bacteriostatic test.

"Denco" cement

An investigation on the use of lactic acid production by saliva as a means of assessing lactic bacillus acidophilus populations is under way. By this means it is hoped to develop a more accurate method of assessing the number of teeth treated with a given material for the recorded number of weeks.

H.A. Hunter

James J. Hae

August 28, 1951.





APPENDIX "G"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Chemical mechanisms in dental caries

Grantee: Dr. J. J. Rae

Project: DR 1 Amount of Grant: \$960

SUMMARY OF WORK

The paper "Ammonia Production in Saliva" by Ballantyne, Clegg, Rae and Lawford, referred to in our last report, has been published in the Journal of Dental Research, Vol. 30, p. 385, 1951. Reprints of this paper are not yet available. A second paper, also referred to in our last report has been revised and is being submitted to the Journal of Dental Research for publication.

The research on the growth inhibitor for lacto bacillus acidophilus found in bromoform treated enamel has been continued. More ground tooth enamel, the fraction of the dried acetone residue which was insoluble in chloroform and the precipitate obtained by treating the above fraction with sodium hydroxide, were prepared and samples sent to Eli Lilly for bacteriacidal test.

An investigation on the use of lactic acid production by saliva as a means of assessing lacto bacillus acidophilus populations is under way. By this means it is hoped to develop a more rapid and less tedious method of assessing caries susceptibility based on l.b.a. counts.

James J. Rae

August 28, 1951.





## APPENDIX "G"

Interim Report No. 12

Subject: Chemical mechanisms in dental caries.

Grantee: Dr. J.J. Rae      Assistant: Mr. C.T. Clegg

Project: DR 1

Period covered: February 1st 1951, to September 1st, 1951.

The aspect of this work dealing with ammonia production in saliva has resulted in two papers, one of which has been published. It is intended to continue this work in the fall, although it was left in abeyance during the spring and summer months.

Since our last report the following work has been done on the bacteriacidal substance found in ground tooth enamel that had been extracted with bromoform or chloroform. At that time ground tooth enamel, after shaking with bromoform or chloroform and drying, was found to have a bacteriacidal effect on l.b.a.

This treated tooth enamel was then extracted with acetone and the acetone extract evaporated to dryness. When this residue had chloroform added a soluble part and an insoluble residue resulted. The insoluble portion was found to contain the bacteriacidal substance. This material was dissolved in alcohol and alcoholic sodium hydroxide added. The precipitate produced in this way after neutralizing with an alcoholic solution of citric acid was found to have a bacteriacidal effect.

In an attempt to identify and purify this inhibitory material, a calcium salt and a barium were prepared. The amounts produced were so small that subsequent analyses were impractical. Since from our point the bacteriacidal behaviour was the most interesting, samples of the various fractions were sent for testing to Eli Lilly in Indianapolis. As yet no report has been obtained.

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James J. Rae

September 4th, 1951.





## APPENDIX "H"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Part I - An investigation of the mechanism of repair of the supporting structures of the teeth; Part II. Fusospirochetal infection and pre-existing inflammation.

Grantee: Dr. C.H. Best

Project: DR 22

Amount of Grant: \$3650

#### SUMMARY OF WORK

Two promising approaches for the development of a clinical procedure in the treatment of periodontal disease are being investigated experimentally by means of periodontal surgery in dogs.

A "surgical" approach is being investigated by means of experimental studies of the use of the periosteum and other connective tissues in reattachment and regeneration of the supporting structures of the teeth. An attempt is being made to determine the cells that are required in the reparative process. Tissues to be examined - 12 blocks - 360 sections.

As part of the surgical investigation, a study of the role of epithelium and epithelial during growths in studies on reattachment is being continued. The results of experimental studies indicate that the down growth of the epithelium is a result of the failure of reattachment and not the cause of the failure.

Tissues examined 6 blocks - 180 sections.

A "physiological" approach is indicated by the suggestion, from the clinical findings in experimental studies on dogs that the ability of the periodontal structures to repair may have a place in the physiology of these tissues.

A study of reattachment and regeneration of alveolar process following the surgical production of epithelialized periodontal pockets in dogs which were allowed to become infected is being carried out. Deep infected, epithelialized, periodontal pockets were produced in 12 dogs. Thirty-four pockets in all were studied with healing intervals of from 9 to 21 months.

Tissues to be examined - 34 blocks - 1000 sections.

This phase of the investigation of the mechanism of repair of the supporting structures of the teeth has necessitated extensive, detailed histological examination of the tissues concerned.

August 31, 1951.

C. H. Best





## APPENDIX "H"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: PART I - An investigation of the mechanism of repair of the supporting structures of the teeth; PART II - Fusospirochetal infection and pre-existing inflammation.

Grantee: Dr. C.H. Best

Project: DR 22                      Amount of Grant: \$3650

#### PART I

An investigation of the mechanism of repair of the supporting structures of the teeth -

As reported to the Committee the findings to date in this research indicate that there are two promising approaches for the development of a clinical procedure in the treatment of periodontal disease; i.e. a surgical approach and a "physiological" approach.

#### A. Surgical approach

In the experimental studies on reattachment and regeneration of the alveolar bone, utilization of the periosteum was made but it is not known whether such repair following detachment and removal of alveolar bone by surgical means was brought about through proliferation and differentiation of cells from the periosteum of from the other tissue present such as the connective tissue of the gingiva, the margins of the bone and the endosteum.

Before a surgical clinical procedure can be developed through studies of experimental reparative processes, the source of cells concerned must be known.

Various surgical procedures all similar to those cited in the studies on reattachment and regeneration of alveolar bone have been carried out in dogs in order to determine the necessary cells required in the reparative processes concerned.

The basic surgical procedure is the flap operation in which the periosteum is lifted with the connective tissue of the gingiva. This is followed by making a defect in the bone and carrying this through to the dentine surface of the root before the flap is sutured into place.

Modifications of the basic procedure were incorporated into the technique to influence the cambium or osteogenic layer of the periosteum.

These were as follows:

- (1) A vigorous scraping of the inner surface of the flap. Two teeth - healing period; 41 days.  
Clinical reattachment was obtained.



(2) Double operation

- (a) Basic surgical procedure - Two teeth - interval of 46 days; clinically reattached.
- (b) Second operation - inner surface of flap more vigorously scraped. From one flap, an attempt was made to remove a thin slice of tissue - healing period 49 days.

In the case of the sliced flap very little reattachment was apparent. The other was reattached.

- (3) Double Operation Control for (2) the flaps were not scraped at second operation - Two teeth - intervals of 42 and 30 days.

Clinical reattachment observed in both cases.

(4) Double Operation

- (a) First flap operation - same as (1) Two teeth - interval 54 days - clinically reattached.
- (b) Second flap operation - inner surface scraped; healing period 113 days.

Both cases were clinically reattached.

As these operations were carried out to study the effect of vigorous interference with an attempted removal of the osteogenic layer of the periosteum, it is hoped that the clinical findings will be supported by the histologic evidence when the sections of these 8 blocks of tissue have been prepared and examined. Serial sections cut at 0.008 mm., 0.25 mm. apart are being prepared. Roughly 250 sections will have to be examined and the findings recorded. Special note must be made of the cellular details.

In the studies on regeneration of alveolar bone, there has always been doubt as to the source of the osteogenic cells which appear around the bone and dentine chips used to fill in the bone defect area. It is probable that they arise from the cambium layer of the periosteum, but one must also consider that they may have grown in from the margins of the bone defect.

To eliminate this possibility, operative techniques were altered slightly so that if the cells came from the margins of the defect, they would have a much greater distance to grow. Usually bone defects have been made about 8.5 x 6 mm. in area. In the following cases the bone defects were made the same distance (8.5 mm.) from the alveolar crest but the defect was carried over almost the whole labial surface of the root of the canine tooth.



Four teeth - flap operation, denuding of labial root surface - filling of defect with bone chips from immediate area - suturing of flap into place.

Thirty-one days later at sacrifice, clinical reattachment was apparent. Histologic examination of sections cut from these four teeth has still to be made.

The development of clinical procedures in the treatment of periodontal disease is complicated by the problem of the epithelial lining of the pocket. Experimental studies already carried out on reattachment and regeneration of the supporting structures of the teeth have indicated that the downgrowth of epithelium is a result of the failure of reattachment and not the cause of the failure of reattachment. Throughout the study there have been cases (mainly lowers) which for one reason or another failed to reattach. Several reasons for the failure of these cases have been put forth. Two outstanding possibilities were: (1) the interference with the operated areas by the dog itself through pawing of the wound which forced hairs and consequent infection deep into the pocket and, (2) the higher muscle attachments in the canine tooth region of the lower jaw of the dog.

(1) An attempt was made to prevent the test animals from interfering with the wounds until healing had occurred by placing a pack over the wound to protect it, and to prevent the insertion of hairs. This technique was carried out on the four lower canine teeth in two dogs.

(a) On one dog, the bone defects were filled with bone chips, the flaps sutured into place (usual bone regeneration technique) and then the operative site covered with pack. These were done at intervals so that when the dog was sacrificed we had data for 12 and 20 days healing periods.

(b) On the other dog, only one of the defects was filled with bone chips. The other defect was allowed to become filled with soft tissue etc. (usual reattachment technique). This animal was sacrificed 63 days after the operation, giving identical healing periods on the two routine techniques.

In each case clinical reattachment had taken place in the two "regeneration" cases, new bone had been deposited in the defects as seen by histologic observation.

Therefore, with the help of a protective covering of pack, reattachment and regeneration had been obtained in the lower canine teeth.



2. In one dog, the muscle attachments of the lower jaw were cut 77 days prior to operation. Then the routine surgical procedures for reattachment and regeneration of alveolar bone were carried out on the two lower canine teeth. The operated areas were covered with pack for protection of the wounds.

After 36 days when the dog was sacrificed clinical reattachment had taken place. Histologic examination revealed the usual successful reattachment and regeneration of the alveolar bone. This experiment increased the number of successful cases among "lower" teeth in the dog.

Thus, through experiment it has been shown that if infection and mechanical stress are eliminated from the healing processes reattachment does occur and downgrowth of epithelium does not take place.

#### B. Physiological Approach

So far, the physiological approach seems to be the most promising. Certain clinical findings in the experimental studies suggest that the demonstrated ability of the periodontal structures to repair may have a place in the physiology of these tissues.

Reattachment following various types of therapy in the treatment of periodontal disease has been reported by many clinicians. A feature common to almost all of the reports is that the evidence of reattachment, largely radiographic, was taken one to five years postoperatively. But in studies already completed it was found that reattachment was apparent in the living animal thirty days postoperatively, and the regeneration of alveolar bone in sixty days when healing occurred by primary union or by a mechanism of repair similar to that in the normal healing of fractures. The time lag between the 60 days and the one to five years in clinical reports suggested the possibility of another slower, and hitherto unknown mechanism of repair. This hypothesis is being investigated by means of a study of reattachment and regeneration of alveolar process following the surgical production of epithelialized periodontal pockets which were allowed to become infected.

The surgical production of periodontal pockets was brought about by covering the exposed root surface of the tooth with periodontal cement pack before suturing the flap into place in the routine flap operation. The cement pack prevents the reattachment of the soft tissues to the denuded root. Twenty canine teeth in five dogs were used in a long term study of approximately 21 months, and fourteen teeth in seven dogs were used in a short term study of from 9 to 33 days.

In these studies, the cement pack was removed after 21 days, an interval which was considered long enough to permit epithelialization of the soft tissue wall of the pocket. The average pocket depth at this time was found to be 10.5 mm. measured from the original gingival crest line to the base of the bone lesion.



After three months, in the long term study, the pocket contents were examined microscopically and found to contain the typical fusospirochetal complex of organisms. At this time the pockets were given one periodontal cement pack treatment and became clinically sterile after 24 hours.

Eighteen months later, the dogs were sacrificed. Measurements of the pockets indicated that there had been reattachment in the cases ranging from 1 to 7 mm., with an average of about 5 mm. The blocks of tissue were removed for serial section.

Some of the sections have been cut and examined but no conclusions have as yet been drawn from the histological findings. A total of roughly 1000 sections must be examined and a record made of cellular details.

The sections from the cases in the short term study will also serve as experimental evidence concerning the downgrowth of epithelium. An understanding of the character and rate of downgrowth of epithelium in cases which have failed to reattach, and in those in which early reattachment was prevented is essential.

It is hoped that this investigation will be completed and a full report ready at the next meeting of the Committee.



## PART II

A final report for period ending June 30, 1951.

This work was carried out in the Banting and Best Department of Medical Research, University of Toronto, by D.C.O'Connell, M.S.A., Research Assistant.

Object: The object of this research was further knowledge of predisposing factors in fusospirochetal infections, and was carried on in conjunction with the main problem of DR 22. It might be said here that throughout the studies carried on by Dr. Linghorne, a careful check was kept on the fusospirochetal infection encountered during the research. It was felt that some understanding of the role that infection played in the study of reattachment and regeneration of the supporting structures of the teeth was necessary.

### Introduction

Experimental fusospirochetal infection provides a means of studying the factors which influence or may have a predisposing effect upon this type of infection associated with periodontal disease.

The experimental infection is produced by parenteral injection of material from disease processes in the mouth which contains the fusospirochetal complex of organisms.

### Progress

A study of experimental fusospirochetal infection of normal guinea pigs, rats and rabbits has been made and reported earlier.

Investigation of the effect of such factors as vitamin deficiencies, diabetes and the products of inflammation of fusospirochetal disease has been made by means of the experimental method previously described.

A more complete study of experimental fusospirochetal infection in normal and vitamin B deficient rats is reported in a paper prepared for publication which will be submitted to the publications committee.

No further work on the effect of a vitamin C deficiency on experimental fusospirochetal infection in guinea pigs has been done. Preliminary experiments described in the last report indicated that scorbutic guinea pigs would sustain a more severe form of experimental fusospirochetal disease than would normal guinea pigs when relatively small doses of infective materials were injected into the subcutaneous tissues.

Experiments planned to investigate the effect of diabetes on experimental fusospirochetal infection in rats and rabbits have not been carried out. Neither time nor cage facilities were available in the first quarter for the commencement of these experiments.



Limited investigation of the effects of the products of inflammation from fusospirochetal disease processes on experimental fusospirochetal infection has been carried out. The very nature of this investigation necessitates detail study of the factors concerned, the use of large numbers of animals and careful study of the results obtained.

### Trauma

The findings throughout this study on experimental fusospirochetal infection have led us to believe that traumatization of the tissues may hasten to a slight degree the progress of infection in experimental animals but it is not essential to initiate fusospirochetal lesions in normal animals. Experimental lesions have been routinely produced in normal guinea pigs, rats and rabbits by the injection of fusospirochetal material from around the teeth without the application of trauma by means of crushing the tissue at the site, movement of the needle or burning of the tissues along the tract of injection. Other workers in the field have found it necessary to apply trauma in order to initiate infection but throughout this study we found that our inocula were sufficiently infective to produce disease without assistance such as the provision of dead or **dying** tissues. The details of an experiment illustrating our findings were given in a previous report.

### Exudate

Preliminary experiments dealing with fractions of the fusospirochetal exudates taken from disease processes in the mouth and from experimental lesions in laboratory animals have been carried on. Actually no real progress along these lines has been made but the results obtained so far are very interesting.

Whole fusospirochetal exudate contains living and dead fusospirochetal organisms, cellular debris, white blood cells, red blood cells (sometimes) and necrotic elements of body tissue, and these are suspended in a fluid made up of inflammatory lymph, and the products of cell disintegration. With some difficulty the solid and liquid fractions of exudate can be separated but at no time has a sizeable amount of the liquid fraction been obtained for experimental use.

### Solids

From a pooled sample of exudate taken from several cases of chronic periodontitis, a portion was removed, centrifuged, and the solids washed in normal saline. The supernatant, in this case being mainly diluent added at the time the exudate was obtained, was discarded. One half of the sediment was resuspended in sterile exudate filtrate, previously prepared, care being taken to maintain the original ratio of solids to liquid. Similarly the other half was resuspended in sterile normal saline.



These preparations were now injected subcutaneously into guinea pigs and rats in 0.4 cc. amounts. Typical experimental fusospirochetal lesions developed in all of the animals and these did not differ in any way from the lesions produced in control animals injected with the untreated exudate.

### Liquid

The supernatant fraction from a sample of guinea pig exudate centrifuged at 2000 r.p.m. for 30 min. was passed through a Seitz filter pad, in a glass filter stick prepared in the laboratory. Even by this method, only a few cc's of sterile filtrate were obtained.

The filtrate was injected in 0.1 cc. amounts into the subcutaneous and dermal tissues of guinea pigs. These small amounts gave rise to only very slight tissue reactions, mainly erythema. No acute inflammatory responses were observed.

### Heat-killed exudate

Because similar lesions resulted from the injection of the organisms and other solids suspended in saline, it was considered necessary to investigate further the "organism" fraction of whole fusospirochetal exudate.

A sample of exudate from a typical guinea pig lesion was heat-killed in a water bath at 60° C for one hour. Darkfield examination of this material showed no motility and cultivation on blood agar both aerobic and anaerobic was negative after seven days.

Two guinea pigs each were injected subcutaneously with 0.1 and 0.05 cc. of the killed exudate and two guinea pigs each were injected intradermally in shaved areas of the back with 0.1 and 0.05 cc. of the material. Control areas in the same animals were similarly injected with normal saline.

Twenty-four hours after the subcutaneous injections, small nodular formations had appeared. These became slightly larger and firmer in 48 hours and 72 hours; but by 96 hours had become small hard fibrotic inflammatory nodules. These findings were verified on autopsy of the animals on the seventh day. The control areas showed no evidence of nodule formation.

Shortly after the intradermal injection of the killed exudate, the triple response skin reaction appeared. Within 2 hours the area at the site was red and edematous. The wheal began to disappear in about 3 hours and inflammatory responses which began after 30 min. became more noticeable. Blood appeared under the skin and the sites of injection became centres of necrosis within 24 hours.



Twenty-four hours after injection all redness due to the H-reaction was gone but inflammed areas persisted with some edema. At 48 hours the necrotic centres were marked but not large. Some edema persisted. At 72 hours the necrotic area has either dried or sloughed away. Practically no edema was present.

The injection of saline into the control areas of skin exhibited the triple reaction but this disappeared within a few hours leaving but slight inflammation.

The heat-killed exudate evidently contains an inflammatory and necrotizing agent which is relatively mild in effect but is capable of causing a positive localized reaction. All animals reacted similarly in this experiment.

## Equipment

### Dry-Ice Chest

Routine storage and long interval preservation of infective exudates was made possible by keeping the exudates in 1 or 2 cc ampules in a dry-ice chest maintained at a temperature of 70°C.

The design and construction of the chest made especially for use in this laboratory is described below.

The chest is constructed of 1/4 inch per plywood. It consists of an upright ablong box 38 x 22 x 22 inches in size. Inside the box is a chamber 12 inches square and 30 inches deep. Between the inner chamber and outer walls of the cabinet there are 4 inches of interlocking sheets of styrofoam insulation (provided by Rowe Packing Company, Toronto).

The inner chamber is divided into two sections. The upper section for dry-ice and the lower section houses a copper cabinet in which are held 10 drawers. Access to the ice chamber is by means of an insulated lid on the top of the chest, and access to the lower section by means of an insulated latched door.

When in use, a 50 lb. block of dry ice (10 x 10 x 10 inches) rests on a supporting frame immediately above the set of drawers. The two inch air space allows circulation of the cold air all around the copper cabinet.

The copper cabinet holds 10 easily removable drawers 6 x 3 x 3 inches in size. Each drawer will hold a maximum of 72, 1 or 2 cc. ampules in 12 rows of 6 each, or a smaller number of larger containers.

To facilitate the use of the dry ice chest each drawer was filled with 7/16" dowel in rows separated by metal dividers. When an ampule was placed in the drawer it took the orderly place of one dowel.

This chest was relatively light in weight, of strong construction, and very economically maintained. A maximum of 100 lbs. of dry-ice per week assured a temperature of 70°C. even in hot weather.

Two views of this chest are shown (see attached two photographs).



Through the use of the dry-ice chest, preservation of infective exudates has been made possible. If this was not the case it would be extremely difficult to develop, maintain and transfer straight line transfer exudates. With the need for infective material to be on hand at all times in order to carry out the experiments with which we have been concerned, the dry-ice chest has proved to be a most valuable piece of equipment.

To illustrate the usefulness of preservation by means of dry-ice, an account of successful preservation of fusospirochetal material is given.

- (a) Gingival scrapings from a case of necrotic periodontitis were used to develop fusospirochetal infection in guinea pigs. Exudates from the resulting lesions were preserved in the dry-ice chest. Samples of these exudates were removed from the chest and thawed in a water bath at 37°C. at intervals of 82 days and 14 1/2 months. In both cases, following subcutaneous injection of the exudate, typical fusospirochetal lesions developed in guinea pigs.
- (b) Material taken from around the teeth and gums of a case of Vincent's infection was used to initiate a strain of fusospirochetal infection in guinea pigs. Exudates from experimental lesions were preserved in the usual way and then removed from the ice chest at intervals of 8 days and 10 months. In each case, the typical fusospirochetal lesions developed after subcutaneous injection of the preserved infective exudate.
- (c) Gingival scrapings from 6 cases of necrotic periodontitis were pooled and mixed well. This material after subcutaneous injection gave rise to typical first passage experimental fusospirochetal lesions in six guinea pigs and eight rats. Collected exudates were frozen and then removed from the chest at intervals of 6 days and ten months and injected into guinea pigs and rats. Typical lesions developed in all animals.
- (d) Gingival scrapings from the mouth of a very old diabetic dog with necrotic periodontitis were injected into four guinea pigs. Typical first transfer lesions developed in the four animals. The remainder of the gingival scrapings was preserved in ampules in the dry-ice chest.

After 32 days one sample of scrapings was removed, thawed and injected into guinea pigs. Typical lesions were found in these animals at autopsy on the sixth day. However, when another sample of the same material was frozen 16 months



before injection into guinea pigs, typical lesions failed to develop in four out of four animals. When the original material was passed once before freezing and then stored for 26 days at 70°C. before further use typical lesions developed following subcutaneous injection of 4 out of 4 guinea pigs. However, once again when an interval of 16 months was allowed before use only 2 out of 4 guinea pigs developed typical lesions. This same material when transferred directly from animal to animal for two and three passages became more stable and produced typical lesions in guinea pigs regardless of the interval allowed between freezing and injection. It is evident that this particular exudate was somewhat unstable to begin with but became more viable as transfer was carried out. Unfortunately insufficient amounts of the other strains of exudate did not allow for freezing of the original gingival scrapings.

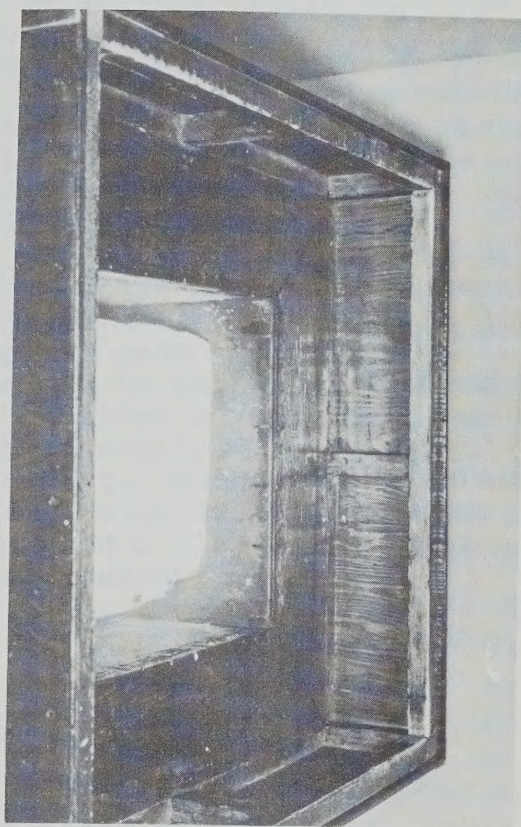
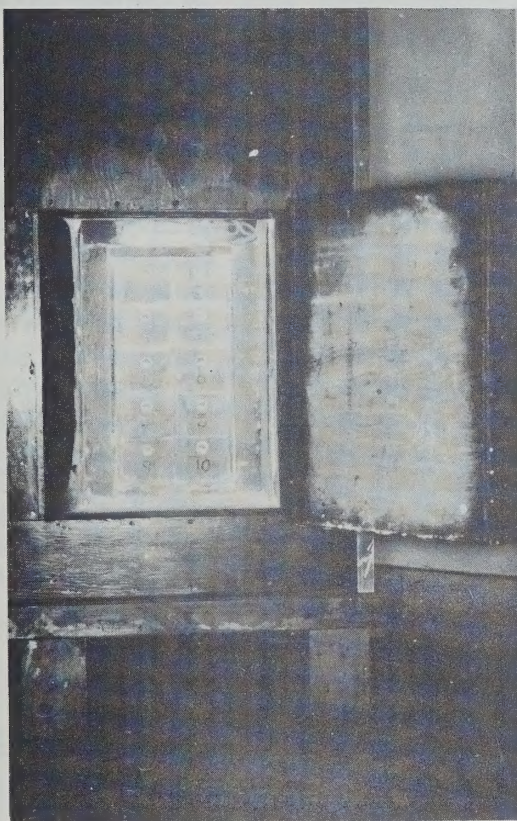
It is possible to initiate experimental lesions in guinea pigs and rats with material from around the teeth and to preserve the lesion exudates in small ampules in the dry-ice chest at a temperature of 70°C., and to be reasonably certain that these preserved exudates will give rise to experimental lesions in other animals after injection regardless of the interval of freezing allowed.

After 35 days one sample of scrapings was removed, thawed and injected into guinea pigs. Typical lesions were found in these animals at autopsy on the sixth day. However, when another sample of the same material was frozen 16 months



"H-13"

DRY-ICE CHEST







## APPENDIX "I"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Entry of phosphate and carbonate salts into teeth as shown with the help of radio-phosphorus and radiocarbon.

Grantee: Dr. C. P. Leblond

Project: DR 23

Amount of Grant: \$2400

#### SUMMARY OF WORK

The work carried out this year has involved a considerable rearrangement of the previous plans. Relatively little work was done with radio-phosphorus for the following reasons: In the past years, several series of experiments have been done to study the entry of radio-phosphorus into the tooth of hamsters and rats up to about the age of 14 days (Bélanger and Leblond, Proc. Soc. Exper. Biol. and Med., 1950, 3: 390). Unfortunately it was not possible to carry out studies beyond that age, since the teeth become too hard to section by the celloidin method which we were then using.

It was mentioned in the previous report that we were working on a method for sectioning the teeth of animals older than 14 days. Dr. Bélanger as well as most laboratories, uses a grinding machine for this type of work. We finally decided to employ an altogether different approach here. With ground sections a very small number of sections can be made, one per tooth generally. If ground irregularly or too thin, the material is lost. Furthermore, grinding is time-consuming. The method devised here by Mr. M. Weiss was derived from that of H.F. Atkinson who used a high speed section cutting machine, (British Dental Journal, January 20, 1951).

The second part of this report is devoted to new results with radio-carbon.

#### 1) Preparation of section of adult teeth

The technique required two steps, first an embedding of the tooth and secondly, the slicing of the tooth sections:

1. Embedding. For embedding, the tooth was impregnated with bioplastic. The technique for this is well known and results into the inclusion of tooth and surrounding soft tissues in a hard, transparent plastic. The main advantage of this technique of embedding is that the bioplastic may be sliced with a steel saw or an abrasive wheel without distortion of the material. Furthermore, this method is very rapid since the time taken from the sacrifice of the animal to completion of the embedding can be reduced to 6 hours.



2. The slicing technique. The plan was to use a mechanism similar to the meat slicer using, however, a rapidly revolving, very thin wheel and a fine micrometer feed taking the plastic embedded tooth to the wheel. The abrasive wheel was chosen because the enamel of teeth is too hard for steel saws and at any rate cutting was much faster. Of the various types of abrasive wheels the rubber bound one was chosen since it was the only one in which the carborundum particles did not become impregnated with the plastic (the others having their cutting edges dulled by the plastic). Furthermore, it was the only type of wheel available as thin as 150 micra. Experience showed that a smooth, clean cut, free from burr and discoloration could be obtained.

As a mechanism, we adopted the Black and Decker Valve Resurfacing Machine (used for surfacing the valves of automobile engines). This machine has cross slides running at right angles to one another, one for feeding the cutting edge to the block to be sliced, the other acting as a micrometer feed and running perpendicular to the side of the wheel. The latter controls the thickness of the slice. The spindle revolves 12,000 times per minute, without creating any vibration. Also this mechanism includes a cooling device with a pump to keep the wheel and plastic at room temperature.

There is still a considerable amount of work to be done in order to obtain as thin a section as possible. Even so, 50 micron-sections were readily obtained. This might be used in some of our problems, but we hope eventually to obtain 25  $\mu$  sections. This thickness would probably be more suitable for the present work. It may be noted that 50 microns thick sections may be further ground by hand polishing. (This operation added a half hour to the procedure).

## 2) Entry of radioactive carbonate into the teeth of new born rats

It may be recalled that, in radioautographs of very young hamsters and rats, we have seen that very soon after administration of radio-phosphorus a dark band appears on the inner surface of the dentine while the pre-dentine shows no reaction. Later the dark band moves outward as new layers of non-radioactive dentine were added to this inner surface.

In work done with Mr. R. Greulich radioautographs of new born rats treated with radio-carbon were obtained at 4, 24, and 72 hours following injection. The teeth were then decalcified, sectioned and radioautographs were made. This material was exposed for eight months and then developed and fixed. At four hours after administration the radioautographs showed a dark band appearing over the pre-dentine close to the tips of the odontoblasts (Figs. 1 and 2). No reaction was visible in the dentine. At 24 and 72 hours the black band was farther and farther away from the odontoblastic surface. In fact at 72 hours the band was visible inside the dentine (Fig. 3). It is suggested that these results make it



possible to visualize the formation of the ground substance of dentine and furthermore that this ground substance is synthesized near the apex of the odontoblasts, that is, in the deeper layers of the pre-dentine. Since the same band is found later in the dentine it is concluded that pre-dentine is in some way a precursor of dentine, a fact which has indeed been suspected in the past but not proved.

A similar band appears in the pre-enamel soon after administration of radio-carbon (Fig. 1) and later moves inward into the enamel (Fig. 2) by apposition of non-radioactive material on the outer or ameloblastic surface of the enamel. The results were, however, not clear cut as with dentine.

These results show that, if we take the phenomena observed after administration of radio-carbon as indicating the fate of the ground substance and those observed after administration of radio-phosphorus as indicating the formation of the calcified material of enamel and dentine, it may be seen that these two processes are quite distinct in timing and location. It has often been thought that odontoblasts secreted both the ground substance as well as the enzyme controlling the precipitation of the tooth salts. The present results indicate that the formation of the ground substance of either dentine or enamel involves a different mechanism than does the formation of the tooth salts.



Legend of the Figures

PLATE 1

Explanation of Figures

The figures 1-3 are coated autographs of the developing incisors of newborn albino rats sacrificed at 4 and 72 hours following injection of  $C^{14}$ . Legend: EP, enamel pulp; IE, intermediate zone of enamel organ; A, ameloblastic epithelium; E, young enamel; D, dentin; PD, predentin; O, odontoblastic epithelium; DP, dental pulp.

1. Incisor, stained with hematoxylin-eosin. Animal sacrificed at 4 hours (large dose of  $C^{14}$ ).

The blackening indicative of an autographic reaction predominates on the newly formed pre-dentine immediately superficial to the tips of the odontoblasts. The separation of odontoblast and ameloblast layers is an artefact caused by preparation of the specimen. Only a slight reaction is present over the ameloblastic epithelium, more marked at its inner surface. The intermediate zone of the enamel organ shows a definite reaction in the form of a fine dark line along the base of the ameloblastic layer. Dental pulp and enamel pulp give a diffuse autographic reaction.

x 135

2. Incisor, stained with hematoxylin-eosin. Animal sacrificed at 4 hours (small dose of  $C^{14}$ ).

As in Figure 1, the autographic reaction is fairly well limited to the area of newly formed pre-dentine particularly that portion adjacent to the odontoblasts. Again, some reactivity is found at or above the apices of the ameloblasts, presumably in the young enamel.

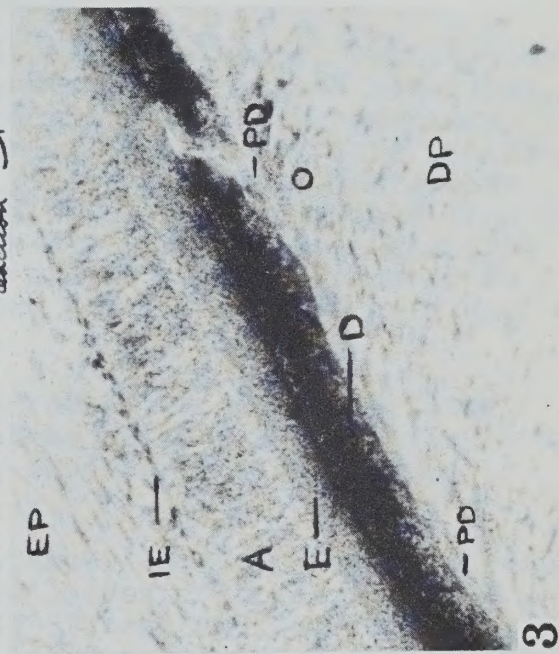
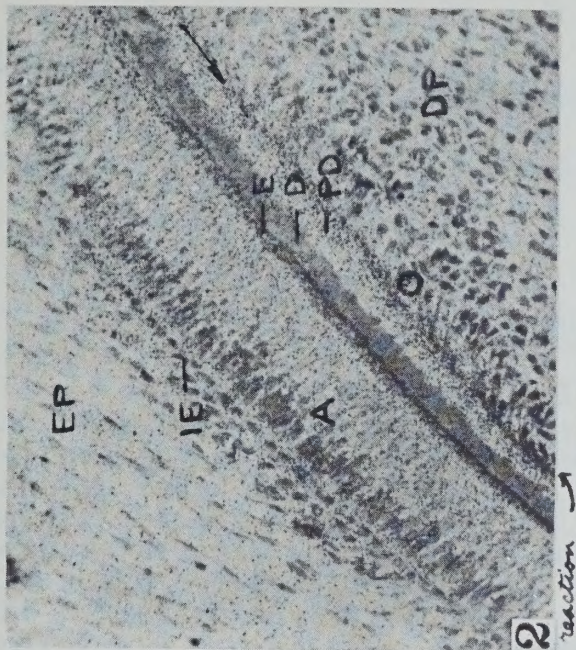
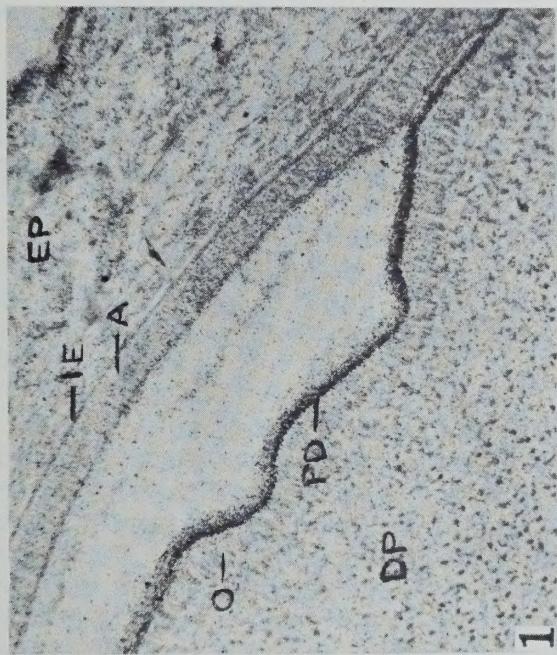
x 225

3. Incisor, stained with hematoxylin-eosin. Animal sacrificed at 72 hours. (large dose of  $C^{14}$ ).

The predominate autographic reaction lies above the definitive dentine, pre-dentine no longer shows any reaction. Enamel gives a diffuse and much lighter reaction. The diffuse reaction of dental and enamel pulp is much paler at 72 hours.

x 245









APPENDIX "J"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1951

Subject: Experimental fusospirochetel infection with mixtures of pure cultures

Grantee: Dr. J.B. Macdonald

Project No.: DR 35 Amount of Grant: \$2,800

SUMMARY

Experiments have dealt with isolation, cultivation and characteristics of motile anaerobes of mucous membranes. Methods investigated for the isolation of these organisms have included serial cultivation of exudate in fluid media, bacteriostasis with the use of 3 different dyes in various concentrations, satellite formation on blood agar and finally alternate cultivation in fluid media and on blood agar from the migrating edge of confluent growth on blood agar or in spirochete cup plates. Of these, the last method only has been successful.

Observation of the characteristics of the forms isolated indicate that they include all the morphologic forms normally observed in guinea pig exudate and they may be classified into three groups.

Group I consists of slender actively motile darting vibrating rods which produce low convex colonies which in certain lights appear flat white "fuzzy" and surrounded by a zone of greening. They produce only scanty growth in highly complex fluid media.

Group II consists of slender "fusiform" rods and long filaments. They show a type of motility hitherto undescribed. The cells are flexible and show jerky alterations in contour from straight to curved or bent outlines and back again to straight. Lashing movements around a fixed pole are also seen. Movements of translation are very rare. The discovery of these organisms has important taxonomic implications because the Order Eubacteriales (the true bacteria) are characterized by rigidity of cells. Members of this group produce grey depressions in blood agar. They grow well in a variety of fluid media but show motility rarely except in veal heart ascites broth with added guinea pig kidney extract. Even here motility is seen in only about 50% of the cultures.



Group III consists of thicker curved rods or short spirals showing a sub polar flagellum on the concave surface and exhibiting a rapid tumbling motility. They grow profusely in Difco thioglycollate broth and very poorly or not at all in all other fluid media tried. In veal heart ascites broth long spirals having up to 15 curves have been seen. This observation may account for previous failure to grow large spirilla in pure culture. This Group does not grow on the surface of blood agar plates.

A number of media and ingredients have been investigated for the cultivation of Group I organisms. The best are complex mixtures containing in a heart infusion base various enrichments--ascitic fluid, aqueous guinea pig kidney extract, and acid hydrolyzed casein or yeast extract. Even the best media support only light growth of these organisms.

Experiments have dealt with isolation, cultivation and characteristics of motile anaerobes of mucous membranes. Methods investigated for the isolation of these organisms have included serial cultivation of exudate in fluid media, bacteriostasis with the use of 5 different dyes in various concentrations, satellite formation on blood agar and finally alternate cultivation in fluid media and on blood agar or in the migrating edge of confluent growth on blood agar or in epitheloid cup plates. Of these, the last method only has been successful.

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## APPENDIX "J"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1951

Subject: Experimental fusospirochetal infection with mixtures of pure cultures

Project No.: DR. 35

Grantee: Dr. J.B. Macdonald

As indicated in the last report on this project, efforts have been directed primarily at the elucidation of some of the characteristics of the anaerobic motile rods which appear uniformly in fusospirochetal exudates and which have been particularly refractory to our efforts at cultivation. The experiments reported here all deal with this group of organisms. By various methods a total of 18 strains of anaerobic motile organisms have been isolated from a variety of fusospirochetal processes occurring naturally in the oral cavity of humans or experimentally in subcutaneous tissues of the guinea pig. The data to be reported is grouped under three general headings:

1. Methods of Isolation
2. Improvement of Media
3. Characteristics of motile anaerobes of mucous membranes.

#### Methods of Isolation

##### A. Serial culture in fluid media.

It was observed earlier in this work that "vibrios" appeared to thrive in close proximity to other members of fusospirochetal exudates, particularly cocci. It therefore seemed possible that the cocci might be supplying growth factors for the "vibrios" and that these latter organisms might therefore survive serial culture of whole exudate in fluid medium better than other members of the group due to the capacity of cocci to survive indefinitely through serial passage. This hypothesis was tested by inoculating three different fusospirochetal exudates into tubes of veal heart infusion containing sterile ascitic fluid, aqueous extract of guinea pig kidney and neutralized cysteine (the modified Proske-Sayers medium referred to in an earlier report). The tubes were incubated anaerobically at 37° and examined by dark field illumination after three days. The growths were then subcultured to fresh tubes of the same medium and incubated for another three days. This procedure was repeated through six passages. It was observed in each case that spirochetes had disappeared by the third passage. In one



case (MU) vibrios disappeared by the 4th passage, in a second (CG) vibrios disappeared on the sixth passage. In the third case (HG) vibrios survived all six passages along with cocci and non motile rods. At the end of six passages the HG exudate seemed complete morphologically except for spirochetes and fusiforms which could not be found while CG exudate was complete except for spirochetes and vibrios. The proportions of organisms in both culture mixtures had changed to give a marked numerical predominance to cocci. In view of the changed nature of these cultures it seemed worthwhile to inoculate guinea pigs with them to determine whether or not the cultures had retained their original pathogenicity.

Three animals were inoculated with 1 cc. each of the sixth fluid culture passage of HG exudate and three were inoculated similarly with CG sixth fluid culture passage. As controls 0.1 cc. of undiluted exudate of each type was inoculated into 2 guinea pigs.

One animal of three in each case developed a small abscess. The exudate recovered showed cocci and non motile rods only and the lesions themselves were well walled off and showed little evidence of necrosis. Both exudates were inoculated in 0.1 cc. amounts into fresh guinea pigs but failed to infect them. Three of the four controls developed typical necrotic abscesses which drained before the 8th day and one developed a small lesion which healed by resolution.

This experiment would indicate that the survival of vibrios in fusospirochetal exudates is dependent on something other than the elaboration of growth factors by cocci or the non motile rods which thrive in the environment of a rich fluid medium. It suggests in fact that the presence of these organisms in the absence of the total flora may actually inhibit the growth of vibrios because the latter in pure culture survive indefinite passage in the medium used in this experiment.

## B. Isolation of organisms on blood agar

Attempts have been made to isolate motile rods from over 30 different sources of gingival scrapings or guinea pig exudates. The method employed was to place drops of mixed suspensions on sheep blood agar plates prepared either with extract broth or veal heart infusion broth. Less frequently the suspensions were inoculated directly into the wells of spirochete cup plates. The inoculated plates were examined after 5 to 7 days anaerobic incubation at 37°. In most cases it was found that growth spread beyond the margins of the original site of inoculum. The resultant border of growth appeared usually as a thin lacey or finely stippled translucent



fringe. Sometimes it appeared as translucent smooth slightly raised fan-like projections. It was not possible to predict from the appearance of this fringe what morphologic forms would be present but it seemed reasonable to expect motile forms inasmuch as this growth represented a migration from the original site of inoculum. This proved sometimes to be the case. In many instances actively motile rods were found in this fringe of growth. More often however a variety of non motile rods and filaments were found. The filaments often attained lengths of several hundred micra and it seems likely that they migrated by reason of their remarkable longitudinal growth rather than as a result of motility.

In all cases where a radiating fringe was found subcultures were made to various fluid media or fresh blood agar plates were streaked from these areas. Two morphologic types of motile organisms were isolated by this method.

The first type which will be referred to for the present as Group I appeared in fluid media after 5 days incubation as slender rods varying in length from 2u to about 8u, either straight or slightly curved and with rounded or slightly tapered ends. They appeared singly, in tandems or in short chains. Their most striking characteristic was their motility. Cells darted back-and-forth very rapidly across the field, sometimes in a straight line and sometimes in a circle. The rate of movement was often so fast that individual cells could not be brought into focus by the eye before they would disappear beyond the margin of the field. Flagella could not be visualized but evidence of their presence was gained by the frequent observation of inter-dependent movement of two cells apparently separated by a space of about 4u. Sunlight illumination as described by Piper was used to attempt to visualize flagella.

Colonies of organisms of this Group were thought for sometime to be very undistinctive--usually smooth low convex greyish translucent and less than  $\frac{1}{2}$  mm. in diameter, sometimes with a flat border. This appearance was so similar to many anaerobic organisms which are found in association with this Group that it was difficult to pick them out. It was discovered more recently however that when viewed in bright reflected light striking the plate at an angle of  $45^\circ$  the colonies had an appearance quite different from that observed when the light struck the plate more obliquely. The colonies now appeared as flat grey-white growth less than  $\frac{1}{2}$  mm. in diameter and with a slightly irregular "fuzzy" margin. If the blood was in a good state of preservation (no hemolysis) the colonies usually showed a narrow zone of greening. This appearance is so characteristic in the twelve strains of this group which have been isolated that one could now approach the problem of isolating this type of organism from a mixed flora with much more assurance of success than previously.



The second type of organisms isolated by this technique has been obtained in pure culture three times. These three cultures are referred to as Group II. Their rate of growth is relatively fast, good growth usually being obtained in 2 or 3 days. They are distinctive in their morphology appearing as slender filaments usually tapered and in this respect corresponding to descriptions of fusiform bacilli. Their length varies from as little as 4u to filaments of more than 100u. There are minor differences in the cell morphology of the three strains. One (WT-OZ) is uniformly double-contoured in dark-field illumination and delicately tapered. Its length is usually about 8 to 30u though as with all three, longer filaments are not uncommon. The second strain (B8-3) is more slender often appearing as single contoured threads. Its length averages 8 to 20u. The third strain (MU-3) is similar in thickness to the first but appears to be shorter--perhaps 4 to 12u on the average.

This group exhibits a type of motility which to the best of our knowledge is hitherto undescribed. These organisms are flexible. Long filaments have frequently been observed to bend or straighten by convulsive flexings. Sometimes the flexion is slower. It may pass as a slow wave along the length of the cell. Sometimes the organisms have been observed to slowly tie themselves in a knot and then untie, a procedure which may take about 3 minutes. The most frequent type of motility is a whip-like lashing around a fixed pole as if one end of the cell were fastened to the slide or coverslip. This observation of flexibility has very interesting taxonomic implications because one of the characteristics of the Order Eubacteriales, the true bacteria, according to Bergey is rigidity of cells.

In every case these organisms were grown in fluid media after subculture from the fringe of a confluent drop. When reinoculated on to blood (and perhaps at this stage not pure) all three grew as irregular translucent fine granular flat colonies. After a number of passages through fluid however all three grew as grey depressions in the agar up to 1 mm. in diameter. These organisms then are isolated not by reason of their distinctive colonial morphology because in early passages it is not evident. Rather they are isolated by blind passage of mixed growth from the edge of a confluent drop to fluid medium and back to blood plates. The organisms grow well in veal heart infusion with ascitic fluid and kidney extract. Growth is evident in 48 hours. Purity of these cultures does not seem to be assured until subculture from the typical grey depressions in the agar is made.



It is noteworthy that Henrikson (1948) described three strains of slender anaerobic rods which produced depressions in agar. Only one of these was motile and the motility was of a rapid progressive type not seen in our strains. Some cells however were described as exhibiting a "pendulating movement back and forth". In 1950, Rosebury Macdonald and Clark described gram negative anaerobic threads which produced depressions in blood agar but were non-motile.

Group III is represented by two strains of organisms (VB4 & VB10) which were isolated from colonies growing on streaked blood plates. Since their original isolation in Rosebury's laboratory they have consistently failed to grow on agar surfaces. A third strain also isolated in Rosebury's laboratory from a haze surrounding a spirochete cup plate which has never been grown as a surface colony may belong to the same Group. This strain (VB7A) did not survive when shipped by air from New York to Toronto. It is hoped that it may be obtained shortly. The two strains available grow well and rapidly in Difco thioglycollate broth producing curved cells and spirals with two or even three curves. Both showed a flagellum emanating from the concave surface. The cells are thicker than in the other two groups (about 1u) and have rounded ends. Motility is active and characterized by a rapid tumbling progression in young cultures (24 hours) but is usually absent or feeble in older cultures. One strain of Group I on the other hand retained its motility for 15 days.

Both strains of Group III grow sparingly if at all in enriched fluid media such as Proske Sayers ascites broth with added guinea pig kidney extract. In such media however they tend to grow in spirillar forms. VB4 on occasion has shown as many as 15 regular spirals in fluid medium. With this strain the longer forms have not been motile. VB10 in fluid medium shows up to 5 regular spirals and good motility. This is perhaps the first time long spirilla have been grown in pure culture. This finding of true spirilla in fluid medium may well explain the presumed failure to cultivate long spirilla from fusospirochetal exudates since in media previously used these organisms appear only as commas or relatively short spirals.

#### C. Satellite formation:

On the hypothesis that fusospirochetal exudate might contain growth factors which might be demonstrated to enhance the growth of vibrios blood agar plates were cross streaked with HG exudate and various strains of vibrios (12 strains from Groups I and III). The same strains were also cross streaked against an anaerobic coccus which was found in close association with a vibrio. Controls consisted of blood agar plates streaked with the various vibrios but not cross streaked. The results were completely negative. Cross streaking did not appear to enhance the growth of vibrios and no satellite colonies were observed.



#### D. Bacteriostasis experiments

The bacteriostatic effect on fusospirochetal exudate and vibrios of three dyes in various concentrations added to Proske Sayers ascites broth was investigated. The dyes used were crystal violet, brilliant green and methylene blue. They were added to the medium in final concentrations of 1:1250, 1:2500, 1:5000 and 1:10,000. Two different guinea pig exudates (HG & CG) and one pure culture of vibrios (JV4) were inoculated into each of the four concentrations of each dye and the tubes were incubated anaerobically for 5 days. A control tube of the medium without added dye was also inoculated for each of the exudates and the vibrios. After incubation samples from all tubes were examined under dark field illumination.

On examination the controls of the exudate cultures both showed heavy growth of cocci and various non motile rods. No motile rods were found. It will be recalled that vibrios survived up to six subcultures in the same medium when the tubes were incubated for only three days. Their absence here in five days suggests as did the experiment when exudate was serially subcultured in fluid medium that the vibrios do not survive in a profusion of cocci and other non motile rods when the total flora is not present.

Practically no growth was found in the two highest concentrations (1:1250 and 1:2500) of any of the dyes. The highest dilution of methylene blue (1:10,000) showed heavy growth of cocci and a few non motile rods. Growth in the same dilution of gentian violet and brilliant green was very light--a few cocci and non motile rods. The 1:5000 dilutions were similar to the 1:10,000 dilutions but were lighter in all cases.

The control of JV4 (the Vibrio culture) showed heavy growth of actively motile darting vibrating rods. There was no growth in any of the dye except the 1:10,000 dilution of methylene blue where a few motile rods were found and the same dilution of brilliant green where a few non motile rods were found.

Since vibrios survive and retain motility in only the 1:10,000 methylene blue dilution which supports heavy growth of cocci it can be concluded that this method is not useful in the isolation of vibrios.



### Improvement of Media

A number of media have been tested for their ability to support growth of vibrios. The various media can be assessed on the basis of three factors.

1. Support or failure to support growth
2. Presence or absence of motility
3. Amount of growth

Except in Group III the last factor--amount of growth--has been of no great significance in these experiments because growth has never been really heavy. It has been generally heavier in Group II than in Group I but variations in the amount of growth in both Group I and Group II have been so minor as to not permit useful comparison.

In Group III however Difco thioglycollate broth has supported heavy growth of both strains while in all other media growth if present at all has been very light. Even in the thioglycollate medium growth is erratic tending at times to petre out in two or three successive subcultures. Motility in this group is excellent in young cultures in Difco thioglycollate medium but has rarely been observed in any other medium. Thus in all respects the thioglycollate medium is clearly superior to all other media tried for cultivation of Group III.

Growth of the three strains of Group II has been moderately abundant appearing as a uniform heavy turbidity in several media in which repeated trials have been made. Motility however is a much less consistent feature (see Table I). In the veal ascites broth motility was seen in 10 of 20 examinations. Motility was seen less frequently, in fact rarely in other media.

Group I has been the most difficult to grow and therefore a number of media have been tested for their ability to support growth of organisms within this group.

The first test was aimed at assessing the value of various ingredients used in the Proske Sayers ascites broth. The results are assessed in Table II on the basis of presence or absence of growth and presence or absence of motility. The experiment clearly indicates the superiority of the more complex media. With the exception of cysteine and sodium thioglycollate the value of each added ingredient is confirmed. There is no evident difference however between the base and the base plus cysteine. It is felt that the role if any of both cysteine and sodium thioglycollate is in lowering the oxidation-reduction potential of the medium. The suggestion of value in the addition of sodium thioglycollate to the



medium was not confirmed when the experiment was repeated using only the two best media. The results for both media (PSAFKE cysteine and PSAEKE cysteine Na thioglycollate) were exactly the same. All twelve strains used grew in both media and all but two were motile in both media.

A second experiment was set up to evaluate the addition of yeast extract, acid hydrolyzed casein or both to the PSAFKE medium. The results are summarized in Table III. It will be seen that either yeast extract or acid hydrolyzed casein added to the medium improved the results. Curiously enough when both were added motility seemed markedly impaired. The suggestion of better motility with the hydrolyzed casein than with yeast extract was not confirmed when the experiment was repeated for these two media. On the second experiment motility was found more frequently with the yeast extract than with the casein hydrolysate (9 motile with yeast and 6 motile with casein).

The chief object of these experiments has been the development of a fluid medium which would support reasonably abundant growth of Group I strains. In this respect the experiments have not been successful. While several of the media used will support growth of these organisms with adequate consistency there has not been a single instance in the hundreds of cultures when growth has produced more than a light turbidity of the medium. All the experiments tend to suggest that these organisms are delicate parasites with enzyme systems appropriate only for their parasitic existence and inadequate to support metabolism in the absence of the rich nutritional environment of their normal habitat.

#### Characteristics of Motile Anaerobes of Mucous Membranes

The characteristics of the three groups of organisms as studied to date are summarized in Table IV. From the foregoing and Table IV it can be seen that the 3 groups are distinctly different in the characteristics which have been studied. The forms which have been seen in the pure cultures of these organisms include all the motile forms normally observed in guinea pig fusospirochetal exudates. It is felt that the approach used has been worthwhile in facilitating the recognition, isolation, and cultivation of these organisms. It is further felt that the problem warrants further study before proceeding to the recombination of cultures from exudate. Are these three Groups related? Are there important differences between strains in the same group? Do any of the groups possess pathogenic potential in pure culture? These and other questions can be answered by study of the biochemical, serological, and pathogenic properties of these organisms.



"J-9"

TABLE I

Growth and motility of Group II Organisms in Fluid Media

| Strains | PSAFKE <sup>*</sup> |                   | Hartley broth |          | Difco thioglycollate broth |          |
|---------|---------------------|-------------------|---------------|----------|----------------------------|----------|
|         | Growth              | Motility          | Growth        | Motility | Growth                     | Motility |
| B8-3    | 9/11 <sup>AA</sup>  | 3/7 <sup>AA</sup> | 11/12         | 3/11     | 9/10                       | 2/9      |
| WT-02   | 10/13               | 4/9               | 6/8           | 0/4      | 5/6                        | 0/4      |
| MU-3    | 5/8                 | 3/4               | 4/4           | 1/4      | 3/3                        | 0/3      |
| TOTALS  | 24/29               | 10/20             | 21/24         | 4/19     | 17/19                      | 2/16     |

<sup>\*</sup>

PSAFKE = Proske Sayers broth + ascitic fluid + guinea pig kidney extract

<sup>AA</sup>

9/11 = growth 9 times in 11 inoculations

3/7 = 3 cultures motile of 7 cultures examined



TABLE II

Growth and Motility of Group I Organisms in Various Fluid Media

| Strain                 | PS <sup>A</sup> |                  | PS <sup>A</sup> cysteine |      | PSAF <sup>A</sup> cysteine |      | PSKE <sup>A</sup> cysteine |      | PSAFKE <sup>A</sup> cysteine |       | PSAFKE cysteine thioglycollate |       |
|------------------------|-----------------|------------------|--------------------------|------|----------------------------|------|----------------------------|------|------------------------------|-------|--------------------------------|-------|
|                        | Growth          | Mot.             | Growth                   | Mot. | Growth                     | Mot. | Growth                     | Mot. | Growth                       | Mot.  | Growth                         | Mot.  |
| JV1                    | +               | 0                | +                        | 0    | +                          | 0    | +                          | +    | +                            | +     | +                              | +     |
| JV2                    | +               | 0                | +                        | 0    | +                          | +    | +                          | +    | +                            | +     | +                              | +     |
| JV3                    | +               | 0                | +                        | 0    | +                          | 0    | +                          | +    | +                            | +     | +                              | +     |
| JV4                    | 0               |                  | 0                        |      | +                          | +    | +                          | +    | +                            | +     | +                              | +     |
| JV5                    | +               | 0                | +                        | +    | +                          | 0    | +                          | +    | +                            | +     | +                              | +     |
| JV7                    | +               | 0                | +                        | 0    | +                          | +    | +                          | +    | +                            | +     | +                              | +     |
| VB2                    | 0               |                  | 0                        |      | 0                          |      | 0                          |      | +                            | 0     | +                              | +     |
| VB5A                   | 0               |                  | 0                        |      | 0                          |      | 0                          |      | 0                            |       | +                              | 0     |
| VB6                    | 0               |                  | 0                        |      | 0                          |      | +                          | 0    | +                            | 0     | +                              | 0     |
| VB8                    | +               | 0                | 0                        |      | +                          | 0    | +                          | +    | +                            | +     | +                              | +     |
| VB9                    | +               | 0                | 0                        |      | +                          | 0    | +                          | +    | +                            | +     | +                              | +     |
| VB11                   | +               | 0                | +                        | 0    | 0                          |      | +                          | 0    | +                            | +     | +                              | +     |
| VB11A                  | 0               |                  | 0                        |      | 0                          |      | +                          | 0    | +                            | +     | +                              | +     |
| Total showing growth   | 8               |                  | 6                        |      | 8                          |      | 11                         |      | 12                           |       | 13                             |       |
| Total showing motility |                 | 0/8 <sup>A</sup> |                          | 1/6  |                            | 3/8  |                            | 8/11 |                              | 10/12 |                                | 11/13 |

PS = Proske Sayers base

AF = Ascitic fluid

KE = guinea pig kidney extract

<sup>A</sup>

0/8 = In 8 cultures showing growth, none motile



TABLE III

Growth and Motility of 11 Strains of Group I Organisms  
in Various enriched media

|          | 1.<br>PSAFKE | 2.<br>PSAFKE yeast ext. | 3.<br>PSAFKE ac. hyd. cas. | PSAFKE yeast ext.<br>ac. hyd. cas. |
|----------|--------------|-------------------------|----------------------------|------------------------------------|
| Growth   | 7            | 11                      | 10                         | 10                                 |
| Motility | 5            | 5                       | 8                          | 1                                  |

1. PSAFKE = Proske Sayers base+ ascitic fluid+ guinea pig kidney extract.
2. 10% of a 10% solution of Difco yeast extract added.
3. 20% of a 20% solution of Difco acid hydrolyzed casein was added.

TABLE IV

Characteristics of Motile Anaerobic Rods . .

| Cell Morphology                                                                   | Motility                                                            | Incubation period | Blood agar                                                         | Difco thiolglyc.                                        | PSAFKE ac. hyd. cas.                                                                            |
|-----------------------------------------------------------------------------------|---------------------------------------------------------------------|-------------------|--------------------------------------------------------------------|---------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Group I<br>Slender rods<br>2-5u long                                              | Rapid darting back-and-forth movement                               | 5 days            | Flat grey-white colonies with "fuzzy" margins and zone of greening | Light floccular growth, usually with motility           | Uniform turbidity with some sediment. Active motility the rule                                  |
| Group II<br>Slender tapered "fusiform" rods of various lengths and long filaments | Pivoting motility on fixed pole. Convulsive flexing of longer cells | 2-3 days          | Grey depressions                                                   | Light floccular growth. Usually no motility             | Moderately heavy uniform turbidity in this and simpler media. Motility frequent but not regular |
| Group III<br>Thick curved rods and spirals with sub polar flagella                | Rapid tumbling progression                                          | 1-2 days          | No growth                                                          | Heavy floccular growth. Good motility in young cultures | Usually no growth or very light. Long spirals found.                                            |



APPENDIX "K"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Fluoride content of enamel and dentin of teeth from Toronto children studied in relation to pabulum history of the children's diets.

Grantee: Dr. M. Doreen Smith

Project: DR 13

Amount of Grant: \$1600

SUMMARY OF WORK

The infant cereal Pablum varies in fluoride content from about 6 p.p.m. to 12 p.p.m. Since some of the new process material has been found to be 12 p.p.m., it appears that the variability in fluoride content is due to the variability of this in dry bone meal mixed with Pablum.

Twenty-four permanent teeth of Pablum-fed children and eleven permanent teeth of non-Pablum-fed children, all between the ages of 5-17 have been separated into dentin and enamel and each fraction analyzed for fluorine. Preparation of teeth, separation into dentin and enamel and analysis of fluorine were done according to the procedures outlined in previous reports.

The results of the two groups are tabulated and the average fluorine content of both dentin and enamel of each group of teeth appear to be the same. This suggests that the fluorine in the Pablum is not assimilated to any great extent into the teeth during childhood or that it is not enough to affect the total fluoride content.

The ratio of the fluoride in dentin to the fluoride in enamel in the teeth analyzed was on the average 1.4 for Pablum-fed children and 1.8 for non-Pablum-fed children.

The results and averages of results of fluoride content after separation and analysis of teeth from Sarnia, Stratford and Brantford are tabulated. The averages for both dentin and enamel from the Sarnia group are lower than from the other two areas.

Date: 7 September 1951.

M.Doreen Smith





## APPENDIX "K"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Fluoride content of enamel and dentin of teeth from  
Toronto children studied in relation to Pablum history  
of the children's diets.

Grantee: Dr. M. Doreen Smith

Project: DR 13                      Amount of Grant: \$1600

The infant cereal Pablum contains an appreciable amount of fluorine as compared with most other cereals. The high fluorine content is due principally to the presence of 2% powdered beef bone. From a number of analyses in this laboratory on various lots of Pablum the fluoride content was found to vary from 6 p.p.m. to about 12 p.p.m. It appears then that the fluoride content of Pablum is not controlled by processing. The variability is probably due to the variability of fluoride content of powdered beef bone. It has been shown that the fluoride content is dependent on the age of the animal and the anatomical source of the bone used for the meal. The amount of fluorine in bone has been reported to vary from 7.69 mgm.% to 21.5 mgm.% (1).

Permanent teeth of children between the ages of 5-17 especially those of the non-Pablum group were difficult to obtain. Twenty-four permanent teeth of Pablum-fed children and eleven permanent teeth of non-Pablum-fed children have been separated into dentin and enamel and each fraction analyzed for fluorine. Preparation of teeth, separation into dentin and enamel and analysis of fluorine were done according to the procedures outlined in previous reports.

The results from the teeth of Pablum and non-Pablum-fed children are recorded in Tables I and II respectively. The average fluoride content of both dentin and enamel of the non-Pablum and Pablum groups of teeth appear to be the same. It is hoped that the numbers especially in the non-Pablum group can be increased in order that the results may be more conclusive. The results suggest that the fluorine in the Pablum is not assimilated to any great extent into the teeth during childhood or that it is not enough to affect the total fluoride content. It has to be kept in mind that the amount of Pablum ingested by children was not known except in two cases. In most cases especially ten or more years ago, feeding of this cereal was often stopped at the end of the first year, or after a few months. A second and more probable conclusion to draw is that the amount of fluorine even if assimilated might not be enough to show when distributed throughout the bones and teeth.

(1) Jackson, S.H. et al, J. Nut. 40, 515. 1950.



The ratio of the fluoride in dentin to the fluoride in enamel in the teeth analyzed was on the average 1.5 for Pablum-fed children and 1.8 for non-Pablum-fed children. A "T" test on these figures showed no significant differences.

The results from the analyses of Sarnia and Stratford and Brantford teeth are shown in Tables III, IV and V respectively. Averages of the fluoride content of dentin and enamel are shown in Table VI. The averages for both dentin and enamel in the teeth from Sarnia are lower than from the other two areas. The averages of the ratios are not very different. The range for the ratios is very great so that the averages on these small numbers of ratio probably means little.

A statistical comparison of the averages of dentin from Sarnia and Stratford teeth showed those from Sarnia were significantly lower in dentin content than those from Stratford at the 1% level.

M. Doreen Smith



TABLE I - Pablum Teeth

| No.        | Age | Sex | Type of Tooth | Degree of Caries | %Fluorine in dentin | %Fluorine in enamel | D/E |
|------------|-----|-----|---------------|------------------|---------------------|---------------------|-----|
| 1          | 7   |     | 1st molar     | severe           | 0.006(2)            | 0.005(9)            | 1.1 |
| 2          | 7   |     | 1st molar     | severe           | 0.008(0)            | 0.003(7)            | 2.1 |
| 3          | 8   |     | 1st molar     | moderate         | 0.005(7)            | 0.005(1)            | 1.1 |
| 4          | 8   |     | 1st molar     | severe           | 0.006(4)            | 0.003(5)            | 1.8 |
| 5          | 8   |     | 1st molar     | severe           | 0.006(8)            | 0.006(8)            | 1.0 |
| 6          | 8   |     | 1st molar     | severe           | 0.007(3)            | 0.005(7)            | 1.3 |
| 7          | 10  |     | L.L.2nd molar |                  | 0.006(6)            | 0.006(6)            | 1.0 |
| 8          | 10  |     | 1st molar     | severe           | 0.010(6)            | 0.006(0)            | 1.8 |
| 9          | 10  | M   | L.1st molar   | severe           | 0.005(9)            | 0.003(1)            | 1.9 |
| #10        | 10  | M   | U.1st molar   | severe           | 0.052(0)            | 0.074(9)            | 0.7 |
| 11         | 10  | M   | L.1st molar   | severe           | 0.006(2)            | 0.004(1)            | 1.5 |
| 12         | 11  | M   | U.2nd molar   | severe           | 0.007(7)            | 0.004(5)            | 1.7 |
| 13         | 11  | F   | L.R.1st molar |                  | 0.008(8)            | 0.009(9)            | 0.9 |
| 14         | 12  | F   | U.R.1st molar |                  | 0.010(2)            | 0.012(6)            | 0.8 |
| 15*        | 12  | M   | 1st bicuspid  | none             | 0.013(5)            |                     |     |
| 16*        | 12  | M   | 1st bicuspid  | none             | 0.012(0)            | 0.011(0)            | 1.1 |
| 17*        | 12  | M   | incisor       | none             | 0.004(6)            | 0.004(2)            | 1.1 |
| 18*        | 12  | M   | incisor       | none             | 0.006(0)            | 0.003(4)            | 1.7 |
| 19         | 13  | F   | L.R.molar     | severe           | 0.006(2)            | 0.003(0)            | 2.0 |
| 20*        | 13  | F   | 1st bicuspid  | none             | 0.010(8)            | 0.006(0)            | 1.8 |
| 21*        | 13  | F   | 1st bicuspid  | none             | 0.008(0)            | 0.004(0)            | 2.0 |
| 22         | 14  |     | bicuspid      |                  | 0.010(1)            | 0.005(1)            | 2.0 |
| 23         | 15  | F   | L. 1st molar  | severe           | 0.005(2)            | 0.003(4)            | 1.5 |
| 24         | 15  | F   | U.R.1st molar |                  | 0.019(4)            | 0.011(3)            | 1.7 |
| averages = |     |     |               |                  | 0.010(1)            | 0.008(8)            | 1.4 |

\* - ate Pablum up to the age of 10 to 12 years.

#Average not including tooth #10 (ashed at too high temperature)

0.008(8) 0.006(3) 1.5



"K-4"

TABLE II - Non-Pabulum Teeth

| No.        | Age | Sex | Type of Tooth  | Degree of Caries | %Fluorine in Dentin | %Fluorine in Enamel | D/E |
|------------|-----|-----|----------------|------------------|---------------------|---------------------|-----|
| 1          | 6   |     | 1st molar      | slight           | 0.009(5)            | 0.002(4)            | 4.0 |
| 2          | 6   |     | 1st molar      | slight           | 0.007(8)            | 0.002(7)            | 2.9 |
| 3          | 6   |     | 1st molar      | slight           | 0.008(0)            | 0.003(0)            | 2.6 |
| 4          | 8   |     | 1st molar      | severe           | 0.006(5)            | 0.006(6)            | 1.0 |
| 5          | 9   |     | 1st molar      | severe           | 0.008(8)            | 0.008(8)            | 1.0 |
| 6          | 9   |     | 1st molar      | moderate         | 0.008(5)            | 0.026(5)            | 0.3 |
| 7          | 10  | F   | 2nd bicuspid   | none             | 0.010(6)            | 0.008(6)            | 1.2 |
| 8          | 10  | F   | 2nd bicuspid   | none             | 0.009(7)            | 0.005(5)            | 1.8 |
| 9          | 15  | F   | 1st incisor    |                  | 0.013(3)            | 0.004(6)            | 2.9 |
| 10         | 17  | M   | U.1st molar    | severe           | 0.011(2)            | 0.023(6)            | 0.5 |
| 11         | 17  | M   | U.1st bicuspid | severe           | 0.012(6)            | 0.005(1)            | 2.4 |
| Averages = |     |     |                |                  | 0.009(7)            | 0.008(8)            | 1.8 |



TABLE III - Fluorine Content of Sarnia Teeth

| No.       | Age | Sex | Type of Tooth | Degree of Caries | %Fluorine in Dentin | %Fluorine in Enamel | D/E |
|-----------|-----|-----|---------------|------------------|---------------------|---------------------|-----|
| 1         | 8   | F   | U.R.1st molar | moderate         | 0.006(3)            | 0.007(3)            | 0.8 |
| 2         | 8   | F   | L.L.1st molar | severe           | 0.006(9)            | 0.007(0)            | 1.0 |
| 3         | 9   | F   | L.R.1st molar | severe           | 0.008(9)            | 0.006(7)            | 1.3 |
| 4         | 11  | M   | L.L.1st molar | severe           | 0.006(8)            | 0.003(9)            | 1.7 |
| 5         | 12  | M   | R.1st molar   | severe           | 0.010(7)            |                     |     |
| 6         | 12  | F   | 1st molar     | severe           | 0.007(8)            | 0.003(2)            | 2.4 |
| 7         | 15  | F   | L.L.molar     | severe           | 0.029(5)            | 0.017(8)            | 1.6 |
| 8         | 16  | M   | L.R.Bicuspid  | severe           | 0.008(1)            | 0.008(7)            | 1.0 |
| 9         | 16  | M   | 1st molar     | severe           | 0.013(0)            |                     |     |
| 10        | 25  | M   | R.U.Cuspid    | moderate         | 0.014(5)            | 0.003(1)            | 4.6 |
| Averages= |     |     |               |                  | 0.011(7)            | 0.007(2)            | 1.8 |

Note - Bracketed numbers show ranges

\* Including previously reported results  
\* If one incisal tooth is omitted

TABLE IV - Fluorine Content of Stratford Teeth

| No.        | Age | Sex | Type of Tooth | Degree of Caries | %Fluorine in Dentin | %Fluorine in Enamel | D/E |
|------------|-----|-----|---------------|------------------|---------------------|---------------------|-----|
| 11         | 14  | F   | Incisor       | none             | 0.030(6)            | 0.018(9)            | 1.6 |
| 12         | 17  | M   | Molar         |                  | 0.027(9)            | 0.014(0)            | 1.9 |
| 13         | 17  | F   | Molar         | moderate         | 0.047(8)            | 0.020(0)            | 2.3 |
| 14         | 21  | M   | Molar         |                  | 0.046(7)            | 0.022(3)            | 2.1 |
| 15         | 25  | F   | Bicuspid      | severe           | 0.075(5)            | 0.039(3)            | 1.9 |
| Averages = |     |     |               |                  | 0.045(7)            | 0.022(9)            | 2.0 |

TABLE V - Fluorine content of Brantford Teeth

|    |   |   |               |        |          |          |     |
|----|---|---|---------------|--------|----------|----------|-----|
| 16 | 8 | F | U.R.1st molar | severe | 0.019(3) | 0.017(9) | 1.1 |
| 17 | 8 | M | 2nd molar     | severe | 0.008(6) | 0.009(7) | 0.9 |

Averages including previously reported results

0.0167 0.0098

TABLE VI - Comparison of Mean Fluoride Content of Stratford, Brantford and Sarnia Teeth

|                        | No. of Teeth | Average % Fluoride in Dentin | Average % Fluoride in Enamel | D/E                 |
|------------------------|--------------|------------------------------|------------------------------|---------------------|
| Stratford              | 5            | (0.027-0.075)<br>0.046       | (0.014-0.039)<br>0.023       | 2.0<br>(1.6-2.3)    |
| Brantford <sup>#</sup> | 11           | (0.009-0.035)<br>0.017       | (0.003-0.032)<br>0.010       | 2.4 (2.1)*(0.9-5.2) |
| Sarnia                 | 10           | (0.006-0.030)<br>0.012       | (0.003-0.018)<br>0.007       | 1.8 (0.8-4.6)       |

Note - Bracketed numbers show ranges

<sup>#</sup> Including previously reported results  
\* If one high-ratio tooth is omitted.



APPENDIX "L"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Development of a method for determining fluorine by means of a photo-electric colorimeter.

Grantee: Dr. O.J. Walker

Project: DR 28

Amount of Grant: \$1000

SUMMARY OF WORK

1. Two papers have been submitted for publication entitled
  - (a) A photometric method for determining small amounts of fluorides.
  - (b) The determination of phosphates by the molybdenum blue method.

The fluoride method discussed in the above paper is the one involving the bleaching of the lake formed by zirconyl salt and sodium alizarin sulfonate by fluorides.

During the Summer considerable work has been carried out on a method involving the bleaching of the aluminum hematoxylin lake by fluorides. The method has been examined critically from the standpoint of pH of solution, proportions of reagents, interference of sulfates and phosphates, time required for developing colour, light filter to be used, and a satisfactory procedure has been worked out. The material is now being prepared for publication.

Some attention has been given to the analysis of teeth and other organic materials where the phosphate content is large enough to interfere with methods for determining fluorides. A rapid method for distilling off fluorides from such mixtures has been found that works satisfactorily. Study of ashing methods for destroying organic material without loss of fluorine is now under way and so far we are not satisfied with any of the methods that have been tried.

The experimental work is being carried out by Mrs. Margaret Jeanne Price.

O. J. Walker

September 11, 1951.





## ASSOCIATE COMMITTEE ON DENTAL RESEARCH

PAPERS PUBLISHEDCommittee  
Paper No.Title and AuthorReference

- |   |                                                                                                                                                             |                                                                                                                          |
|---|-------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| 1 | New aspects of periodontal research,<br>by H.K. BOX                                                                                                         | Presented at National Convention of Canadian Dentists, Toronto, 29 May 1946. (App."C", 5th Mtg. A.C.D.R., 25 Feb. 1947). |
| 2 | Concerning the pathogenesis of periodontal disease,<br>by H.K. BOX                                                                                          | J.Periodontology, 19: 11-20. 1948. (App."G", 7th Mtg., A.C.D.R., 2 Mar. 1948).                                           |
| 3 | The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid,<br>by J.J. RAE and C.T. CLEGG    | J. Dent. Res. 27: 52-53. 1948.                                                                                           |
| 4 | Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate,<br>by J.J. RAE and C.T. CLEGG | J. Dent. Res. 27: 54-57. 1948.                                                                                           |
| 5 | An improved method for processing acrylic dentures,<br>by D.R. CHRISTIE                                                                                     | J. Can. Dent. Assoc., 14: 303-317. 1948. (App."R" 8th Mtg., A.C.D.R., 26 Oct., 1948).                                    |
| 6 | The therapeutic properties of periodontal cement packs,<br>by W.J. LINGHORNE and D.C. O'CONNELL                                                             | J. Can. Dent. Assoc., 15: 199-205. 1949.                                                                                 |
| 7 | Phosphatase in saliva,<br>by J.T. DENTAY and J.J. RAE                                                                                                       | J. Dent. Res., Feb. 1949. 4 pp.                                                                                          |
| 8 | The treatment of acute oral Vincent's infection,<br>by W.J. LINGHORNE and D.F. STEDMAN                                                                      | J. Can. Dent. Assoc., July, 1949. 6 pp.                                                                                  |
| 9 | A comparison of nine local anaesthetics, by<br>H.S. HAMILTON, B.A. WESTFALL and J.K.W. FERGUSON                                                             | J. Pharmacol. Expt. Therap. 94: 299-307. 1948.                                                                           |





| <u>Committee<br/>Paper No.</u> | <u>Title and Author</u>                                                                                                                               | <u>Reference</u>                               |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| 10                             | New methods of repairing acrylic dentures without distortion, by D.R. CHRISTIE                                                                        | J. Can. Dent. Assoc., 15: 582-590. 1949.       |
| 11                             | The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. COGGLES, O.J. WALKER and H.R. MACLEAN                                | J. Can. Dent. Assoc. 16: 75-82. 1950.          |
| 12                             | The relation between buffering capacity, viscosity and lacto-bacillus count of saliva, by J.J. RAE and C.T. CLEGG                                     | J. Dent. Res. 28: 589-593. 1949.               |
| 13                             | Mineralization of the growing tooth as shown by Radio-phosphorus autographs (17692), by L.F. BELANGER and C.P. LEBLOND                                | Proc. Soc. Expt. Biol. Med. 73: 390-391. 1950. |
| 14                             | Radio-autographic visualization of bone formation in the rat, by C.P. LEBLOND, G.W. WILKINSON, L.F. BELANGER and J. ROBICHON                          | Am. J. Anat. 86:2. 1950.                       |
| 15                             | Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH                                                                        | Can. J. Research, F, 28: 227-233. 1950.        |
| 16                             | Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W.J. LINGHORNE and D.C. O'CONNELL | J. Dent. Res. 29: 419-428. 1950.               |
| 17                             | An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNES and M. DOREEN SMITH                                 | J. Can. Dent. Assoc., Jan. 1951. 5 pp.         |
| 18                             | Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J.P. LUSSIER                                                  | Rev. Canadienne de Biol. 10:175-180. 1951.     |
| 19                             | Relining acrylic dentures without distortion, by D.R. CHRISTIE                                                                                        | J. of the Can. Dent. Assoc., July 1951. 4 pp.  |

The Secretary of the Committee has a small stock of most of the papers listed and will supply copies to members of the Committee on request.

September 1951.





## APPENDIX "N"

### REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1951

Subject: A Study of Migration of Tooth Germs During  
and Before Mixed Dentition

Grantee: Willa Liu Chou

Project: Fellowship Grant      Amount of Grant: \$2200.00

#### SUMMARY OF WORK

Investigation of the project has not been started because Dr. Robert E. Moyers, the supervisor of the study went to Europe this summer. In the past two months the investigator has made a general review of the literature on studies of the growth and development of the face and head and the dental tissues by Brodie, Broadbent, Downs, Wiley, Carlson, Hillman etc., and has learned the cephalometrical technic. Method of approach for this study has been worked out. As soon as Dr. Moyers comes back at the end of September, the practical work will be started.

The study will be carried out by using series of lateral, posterior and anterior head X-rays of a child. Tracings of the X-ray pictures will be made and measurements will be taken from certain points on the tooth germs to certain reference planes on the tracing. Analysis of data will be carried out according to sex and classes of malocclusion.

Since there is no series of X-ray records available for this study in the Faculty, it may be necessary to borrow series of head X-rays from University of Iowa or Western Reserve University. The investigator will apply for travelling grant for going down to the States, to select her materials in any one of the above mentioned universities.

September 10, 1951

Roy G. Ellis  
in Dr. R.E. Moyers'  
absence





APPENDIX "O-1"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September, 1951

Subject: Oral Pathology and Bacteriology

Grantee: Dr. Gerald Shklar

Project: Fellowship Grant

Amount of Grant: \$1500

Dr. Shklar has just recently begun work under the fellowship but already has conducted preliminary experiments with animals in an effort to study the role of allergic phenomena in periodontal disease. Many tissue sections have been prepared from the animals and preliminary observations have been made on them. Dr. Shklar is an enthusiastic capable young man with a keen interest in the research aspects of dentistry. I anticipate that he will prove himself worthy of the fellowship he holds.

12 September, 1951

Irving Glickman

(See page "O-2" for report by Dr. G. Shklar)

8 September 1951.





APPENDIX "0-2"

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1951

Subject: Oral Pathology and Bacteriology

Grantee: Dr. Gerald Shklar

Project: Fellowship Grant Amount of Fellowship: \$1500

SUMMARY OF WORK

Allergy has been suggested by many workers in the periodontal field as one of the local etiological factors in the production of gingivitis and chronic destructive periodontal disease. It was suggested by Albanese that the sensitization of the oral tissues to certain bacterial forms may be responsible for an allergic form of inflammatory disease. Several workers have reported a local eosinophilia in the gingival differential blood count in patients with periodontal disease. This was considered an indication of an allergic state.

We are at present conducting experiments to determine whether the introduction of bacterial allergen into sensitized oral tissues of the guinea pig would result in a local inflammatory state. The oral flora of the guinea pig was studied and a characteristically present Streptococcal strain was isolated. Suspensions of the heat-killed organisms were employed as allergen. Local introduction of bacterial allergen into the gingiva of guinea pigs does not appear to produce an inflammatory reaction. However, introduction of the bacterial allergen subcutaneously into the groin of the animals does appear to produce certain gingival changes which are being studied. The reaction may be comparable to the Sanarelli-Shwartzman phenomenon. Controlled experiments are being carried out on rabbits and guinea pigs to adequately assess the possible role of bacterial allergy in the production of oral disease.

G. Shklar

8 September 1951.





## APPENDIX "P"

### Suggested Research Projects

By decision of the Committee (Min.4(x), 13th Mtg.) projects suggested as research topics are listed hereunder for consideration by Committee members:

| <u>Proposed Subject</u>                                                                                                                         | <u>Suggested by:</u> | <u>Date</u> |
|-------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|-------------|
| 1. Gold inlays over amalgams<br><br>Little information is available as to whether gold inlays over amalgams cause disintegration of the cement. | Dr. M.H. Garvin      | Oct. 1950   |
| 2. A study of root canals of teeth<br><br>This is a very practical research subject as the results would be of value to every practitioner.     | Dr. M.H. Garvin      | Oct. 1950   |
| 3. Social aspect of dental disease                                                                                                              | Dr. A.C. Burton      | Sept. 1951  |

September, 1951.





Members of the Associate Committee on Dental Research  
and its Executive, with their terms of appointment

Term to Expire

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